DOCUMENT-IDENTIFIER: US 5139933 A
TITLE: Assay method for detecting listeria

Detailed Description Text (22):
Monoclonal antibodies directed against Listeria cell wall teichoic acids are also produced by methods familiar to those in the field. Immunization is carried out using Listeria cell wall preparations made by the method of Schleifer, K. H. and Kandler, O.; (1967) Arch. Mikrobiol. 57, 35-363. These cell wall preparations contain (TA) covalently linked to the cell wall peptidoglycan matrix. For immunization the peptidoglycan will act as the immunogenic carrier. Greater antibody specificity may be achieved by using purified (TA) as the immunizing agent. For this, the cell walls may be digested chemically (for example with 10 mM glycine hydrochloride buffer pH 2.5). The (TA) is then purified and used as the immunogen. To increase the immune response these purified (TA) may be coupled to an immunogenic carrier protein. For example, the reducing sugar end-group of the digested (TA), as above, may be coupled to a carrier protein by reductive amination using the method of Roy et al. (1984) Canadian J. of Biochem., 62, 270-275, as applied to Listeria (TA) by Kamisango et al. (1985), J. Clin. Microbiol., 21, 135-137. In this procedure (TA) are reacted with a carrier protein such as BSA in the presence of sodium cyanoborohydride. The result is a covalent teichoic acid-protein complex which is used for immunization.

Detailed Description Text (44):
U.S. Pat. No. 4,556,769 noted above describes an assay method using monoclonal antibodies to peptidoglycan. However, the present invention importantly uses as the final detection step the use of labeled antibodies against teichoic acid. Since there are approximately 30 ribitol units per (TA) molecule, and between an equal number and four times the number of (PEP) disaccharide-peptide repeating units as (TA) molecules in each listeriae cell, the assay of the invention permits targeting of about 8 to 30 times as many antigenic sites, thereby enhancing assay sensitivity. Using the (TA) moiety of the PEP-TA complex as the labeled antibodies target also allows for Listeria strain identification, and thus pathogenic vs. nonpathogenic Listeria. This would not be possible if (PEP) alone were used to assay for Listeria strains. In fact Listeria strain identification is not possible if only cell wall (PEP) is used for a target as other gram positive and most gram negative bacteria share the same (PEP) chemical structure as Listeria (Schleifer, K. H. and Kandler, O., (1972) Bacterioloqical Reviews, 36, p. 404-477). Therefore, the antigenic epitopes are essentially the same.

First Hit Fwd Refs

L6: Entry 8 of 11 File: USPT Mar 1, 1994

DOCUMENT-IDENTIFIER: US 5290707 A
TITLE: Method for detection of microorganisms

Detailed Description Text (26):
Bacteria are designated first as gram-positive, by polyclonal recognition of teichoic acid cell wall fraction, or as gram-negative, by polyclonal antibody recognition of lipopolysaccharide (LPS), a gram-negative cell wall component. Further identification of specific pathogenic factors is based on DNA hybridization.

DOCUMENT-IDENTIFIER: US 20030228322 A1
TITLE: Multifunctional monoclonal antibodies directed to peptidoglycan of gram-positive bacteria

Summary of Invention Paragraph:
[0005] Because of the prevalence of these bacteria on the skin and other surfaces, most mammals are exposed to Gram-positive bacteria. Thus, the polyclonal serum from any mammal, including humans, is likely to contain IgG that will bind to many different cell wall and surface components of Gram-positive bacteria. Such a collection of IgGs may serve to protect against Gram-positive bacteria because polyclonal IgG binding to many epitopes on surface antigens or cell wall molecules (such as peptidoglycan, teichoic acid, lipoteichoic acid, proteins and carbohydrates) may collectively be opsonic and promote phagocytosis of Gram-positive bacteria. Thus, the composite function of the antibodies in polyclonal serum may account for the serum's functional activity. However, such polyclonal IgG is clearly not always protective, as evidenced by the continued presence of infections due to such bacteria. To augment the level of antibodies against Gram-positive bacteria, clinicians administer vaccines based on these bacteria. However, many bacterial cell extracts that are used for immunization are not pure for one epitope or antigen, so the activity of the resulting antibodies may represent activities against several different cell wall components. This is particularly problematic if the cell wall is the antibody target, and the purity of the cell wall preparation cannot be verified.

Summary of Invention Paragraph:
[0008] Moreover, until recently, determining the role of peptidoglycan or of antibodies to peptidoglycan was complicated by the impurity of peptidoglycan preparations. Teichoic acids and lipoteichoic acids are closely associated with cell wall peptidoglycan. In addition, for some bacteria, such as S. epidermidis, teichoic acid and lipoteichoic acid have the same glycerol phosphate backbone. These teichoic acid moieties can easily contaminate peptidoglycan preparations, which are prepared from cell wall extracts. Thus, the activity of serum raised against these preparations may not result from the activity of antibodies to peptidoglycan, but instead from the activity of antibodies to contaminates (see, e.g., (36)). Recently, we have developed monoclonal antibodies to LTA that have multiple functional activities, including opsonic activity, against Gram-positive bacteria. These antibodies can be used to confirm that peptidoglycan preparations are free of LTA contamination.

L7: Entry 23 of 30 File: USPT Mar 20, 2001

DOCUMENT-IDENTIFIER: US 6203997 B1
TITLE: Quantitation of analytes in whole blood

CLAIMS:

8. The method of claim 1 wherein said anti-analyte antibody is a monoclonal antibody of class IgM or IgG.

9. The method of claim 1 wherein said analyte is selected from the group consisting of gram-positive bacteria, gram-negative bacteria, fungi, viruses, gram-positive cell wall constituents, lipoteichoic acid, peptidoglycan, teichoic acid, gram-negative endotoxin, lipid A, hepatitis A, inflammatory mediators, drugs of abuse, therapeutic drugs, and cardiac markers.

12. A diagnostic kit for quantitating the level of a preselected analyte present within sample of blood of a human or animal patient, said sample comprising plasma and white blood cells, said diagnostic kit comprising:

i) a first container of IgM or IgG antibody specific to the preselected analyte;

ii) a second container of chemiluminescent compound;

iii) a third container of antigen; and

iv) a fourth container of anti-antigen antibodies.

13. The diagnostic kit of claim 12 wherein said analyte is selected from the group consisting gram-positive bacteria, gram-negative bacteria, fungi, viruses, gram-positive cell wall constituents such as lipoteichoic acid, peptidoglycan and teichoic acid, gram-negative endotoxin, lipid A, hepatitis A, inflammatory mediators, drugs of abuse, therapeutic drugs, and cardiac markers.

DOCUMENT-IDENTIFIER: US 4250262 A
TITLE: Method of preparing a purified glucosyltransferase

Detailed Description Text (62):
Previously we have suggested that two likely antigens of S. mutans for immunization experiments might be either the serotype-specific carbohydrate antigen or the GTF enzymes. Smith, D. J., and M. A. Taubman. 1976. Immunization experiments using the rodent caries model. J. Dent. Res. 55 (Special Issue C):C193. This suggestion was based on the demonstration that antibody directed to either of these antigens had the capacity to interfere with adherence phenomena demonstrated by S. mutans in vitro. Mukasa, H., and H. D. Slade. 1974. Methanism of adherence of Streptococcus mutans to smooth surfaces. II. Nature of the binding site and the adsorption of dextran-levan synthetase enzyme on the cell wall surface of the streptococcus. Infect. Immun. 9:419, Iacono, V. J., M. A. Taubman, D. J. Smith, P. R. Garant, and J. R. Pollock. 1976. Structure and function of the type-specific polysaccharide of Streptococcus mutans 6715. Immunology Abstracts (Special Suppl.):75. Although both antigens occur in culture supernatants, in the current invention, we have been careful to eliminate type-specific antigen from all our GTF preparations used for immunization. Antibody reactive with the type-specific antigen was never detected in the serums of any of the animals immunized with these enzyme preparations. The levels of protection reported in the above examples, utilizing CE-1 and CE-2 GTF as an antigen in the rat models, are quite comparable to the levels of protection obtained previously after immunization with whole cells. Taubman, Smith, supra. The experiment in hamsters with more defined GTF enzymes (DE-1 and DE-2) as immunogens also supports the contention that GTF enzyme is of major importance as antigen. Although the evidence is not unequivocal, there are several additional compelling reasons in support of the case for GTF enzyme: (a) Other enzyme antigens (e.g., fructosyltransferase or invertase) were probably absent from our GTF fractions. (b) Both GTF enzyme preparations, having only one enzyme antigen in common, gave rise to protection. (c) Although the DE-1 preparation contained trace amounts of material reactive with an antiserum directed to the polyglycerol phosphate (PGP) backbone of teichoic acid, as did DE-2, serum antibody from hamsters immunized with either of these preparations did not react with teichoic acid from S. Sanguis. (d) Immunized animals showed antibody in serum and saliva which would bind and inhibit GTF activity. (3) The likelihood of DE-1 and DE-2 containing common antigens other than enzyme is low due to the complex series of procedures followed and the purposeful selection for material demonstrating enzyme (GTF) activity. Nevertheless, it is clear that even more purified enzyme as antigen would better establish the importance of GTF in the pathogenesis of S. mutans and also the importance of GTF as antigen for immunization.

Detailed Description Text (87):
The 2% agarose pool was also examined for antigenic components in immunodiffusion. The GTF pool was placed in the central well. Antisera to the serotype q antigen, to teichoic acid, to the glucan of S. mutans strain 6715, and to a crude enzyme antigen preparation from culture supernatants of the 6715 strain were placed in the outer wells. No serotype antigen or teichoic acid could be detected in the enzyme preparation according to the invention. However, a precipitin band did form with the anti-glucan antiserum. The anti-CEA antiserum reacted predominantly with one component, forming a precipitin band close to the antigen wall. This precipitating system migrated in immunoelectrophoretic analyses to the same region as the water-insoluble synthetic activity, identified in a separate run when sucrose was added to the trough. This indicated that the band seen in gel diffusion against the anti-CEA serum contained GTF. Thus, the guanidine-eluted and gel-filtered GTF pool seems to contain one protein component which is enzyme and one carbohydrate component which is glucan.

# WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Friday, September 16, 2005

| Hide? | Set Name | Query | Hit Count |
|---|---|---|---|
| | | *DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR* | |
| ☐ | L1 | teicho$ near acid$ | 326 |
| ☐ | L2 | L1 and wall | 239 |
| ☐ | L3 | L2 and (polyclonal or poly-clonal or antisera or antiserum or anti-sera or anti-serum or immunoglobulin or globulin or igg or igm or iga or ig or monoclonal or hybridoma or mono-clonal or mab or moab or m-ab or chimeric or humanized or ivig or igiv or iv-ig ivigg) | 177 |
| ☐ | L4 | L1 same wall | 147 |
| ☐ | L5 | L4 and l3 | 103 |
| ☐ | L6 | L4 same (polyclonal or poly-clonal or antisera or antiserum or anti-sera or anti-serum or immunoglobulin or globulin or igg or igm or iga or ig or monoclonal or hybridoma or mono-clonal or mab or moab or m-ab or chimeric or humanized or ivig or igiv or iv-ig ivigg) | 11 |
| ☐ | L7 | L4 and (polyclonal or poly-clonal or antisera or antiserum or anti-sera or anti-serum or immunoglobulin or globulin or igg or igm or iga or ig or monoclonal or hybridoma or mono-clonal or mab or moab or m-ab or chimeric or humanized or ivig or igiv or iv-ig ivigg).ti,ab,clm. not l6 | 30 |
| ☐ | L8 | antiphosphorylcholine | 4 |
| ☐ | L9 | anti-phosphorylcholine | 47 |
| ☐ | L10 | L9 not l8 | 43 |

END OF SEARCH HISTORY

DOCUMENT-IDENTIFIER: US 6159683 A
TITLE: Method of determining stage of sepsis

Brief Summary Text (21):
The microbial products indicative of sepsis include gram-positive bacteria, gram-negative bacteria, fungi, viruses, gram-positive cell wall constituents such as lipoteichoic acid, peptidoglycan and teichoic acid, gram-negative endotoxin, and lipid A.

Brief Summary Text (26):
A wide variety of microbial products may be assessed with the diagnostic kit. These include for example gram-positive bacteria, gram-negative bacteria, fungi, viruses, gram-positive cell wall constituents such as lipoteichoic acid, peptidoglycan and teichoic acid, gram-negative endotoxin, and lipid A. In a preferred embodiment, the diagnostic kit includes an additional container containing an agent capable of increasing oxidant production by white blood cells on exposure to immunocomplexes, for example, zymosan, latex particles, opsonized zymosan, or opsonized latex particles. Opsonized zymosan is preferred.

Detailed Description Text (7):
The microbial product analyte may be selected from any of a number of such products, including gram-positive bacteria, gram-negative bacteria, fungi, viruses, gram-positive cell wall constituents such as lipoteichoic acid, peptidoglycan and teichoic acid, gram-negative endotoxin, and lipid A.

CLAIMS:

2. The method of claim 1 wherein the microbial products are selected from the group consisting of gram-positive bacteria, gram-negative bacteria, fungi, viruses, gram-positive cell wall constituents, lipoteichoic acid, peptidoglycan, teichoic acid, gram-negative endotoxin, lipid A, and combinations thereof.

7. The method of claim 3 wherein said anti-microbial product antibody is a monoclonal antibody of class IgM or IgG.

8. The method of claim 3 wherein said microbial product is selected from the group consisting of gram-positive bacteria, gram-negative bacteria, fungi, viruses, gram-positive cell wall constituents, [such as] lipoteichoic acid, peptidoglycan, teichoic acid, gram-negative endotoxin, lipid A, and combinations thereof.

9. A diagnostic kit for determining the stage of sepsis from a sample of patient's blood comprising:

i) a first container of IgM or IgG antibody specific to a microbial product;

ii) a second container of chemiluminescent compound;

iii) a third container of antigen;

iv) a fourth container of anti-antigen antibodies;

v) a fifth container of tumor necrosis factor; and

vi) a sixth container of an agent capable of increasing oxidant production by white blood cells on

exposure to immunocomplexes or tumor necrosis factor.

10. The diagnostic kit of claim 9 wherein said microbial product is selected from the group consisting of gram-positive bacteria, gram-negative bacteria, fungi, viruses, gram-positive cell wall constituents, lipoteichoic acid, peptidoglycan, teichoic acid, gram-negative endotoxin, lipid A, and combinations thereof.

DOCUMENT-IDENTIFIER: US 20030159166 A1
TITLE: H2-O modified transgenic animals

Brief Description of Drawings Paragraph:
[0020] (B) Presentation of antigens internalized by mIg receptor mediated uptake. B cells from transgenic H2-0.sup.+/+ (m) and H2-O.sup.-/- (s) mice expressing antiphosphorylcholine antibody were pulsed with phosphorylcholine conjugated antigens for 1 hour, then washed extensively to remove the excess antigen and incubated with hybridoma cells overnight. Values represent the mean IL-2 production.+-.SD from triplicate cultures. Levels of statistical significance between the means using students t-test are indicated (* $P<0.05$, ** $P<0.01$, *** $P<0.005$).

Detail Description Paragraph:
[0134] Gearhart, P. J., Sigal, N. H., and Klinman, N. R. (1975). Heterogeneity of the BALB/c antiphosphorylcholine antibody response at the precursor cell level. J Exp Med 141, 56-71.

Detail Description Paragraph:
[0162] Storb, U., Pinkert, C., Arp, B., Engler, P., Gollahon, K., Manz, J., Brady, W., and Brinster, R. L. (1986). Transgenic mice with mu and kappa genes encoding antiphosphorylcholine antibodies. J Exp Med 164, 627-41.

| Search | Most Recent Queries | Time | Resu |
|---|---|---|---|
| #23 | Search **ribitol teichoic acid antibodies staphylococcus** | 13:45:36 | |
| #22 | Search **rta antibodies staphylococcus** | 13:45:11 | |
| #20 | **Related Articles** for **PubMed** (Select **3623839**) | 13:44:37 | 2 |
| #13 | Search **wall teichoic acid antibodies** | 13:36:15 | 1 |
| #11 | Search **2001 lethal toxin protection** | 08:13:13 | |
| #10 | Search **2001 lethal toxin price** | 08:12:39 | |
| #9 | Search **2001 lethal toxin** Sort by: **PublicationDate** | 08:12:29 | 4 |
| #8 | Search **price lethal toxin** | 08:12:07 | |
| #7 | Search **price 2001 infection immunity** | 08:11:50 | |
| #6 | Search **price 2001 toxin infection immunity** | 08:11:42 | |
| #5 | Search **price 2001 toxin** | 08:11:26 | |
| #4 | Search **price liner park leppla 2001 toxin** | 08:11:16 | |
| #2 | Search **park leppla 2000 anthracis lethal factor** | 08:07:56 | |
| #1 | Search **park leppla 2000 anthracis letal factor** | | |

Archibald, Ar et al, Nature-New Biology, Jan. 3, 1973, vol. 241(105), pp. 29-31, Molecular arrangement of teichoic acid in the cell wall of Staphylococcus lactis.

Other Reference Publication (36):
Oshima et al., Comparison of Cell Wall Teichoic Acid Fractions Isolated from Three Different Encapsulated Strains of Staphylococcus epidermidis, Ann. Microbiol. 135:353-65 (1984).

CLAIMS:

0107] 1.5. Effects of Skp Co-Expression on Soluble Protein Expression

[0108] We then determined the effect of Skp on the production of several of the scFv fragments in soluble form using the non-suppressor strain JM83. Using antigen-binding ELISA (FIG. 6) it can be seen that the amount of soluble scFv was dramatically increased in the presence of co-expressed Skp. To demonstrate that this is also reflected in the yield of purified protein, the scFv fragment of the anti-phosphorylcholine binding antibody McPC603-H11 (Knappik & Pluckthun, 1995) was tested. Co-expression of Skp increased the amount of protein, purified by affinity-chromatography on phosphorylcholine by about a factor of 4.

[0140] Knappik, A. and Pluckthun, A. 1995. Engineered turns of a recombinant antibody improve its in vivo folding. Protein Eng. 8:81-89.

L10: Entry 2 of 43 File: PGPB Aug 5, 2004

DOCUMENT-IDENTIFIER: US 20040152103 A1
TITLE: Novel methods for obtaining, identifying and applying nucleic acid sequences and (poly)peptides which increase the expression yields of periplasmic proteins in functional form

Detail Description Paragraph:
[0100] Protein purification. The anti-phosphorylcholine scFv McPC603-H11 (Knappik & Pluckthun, 1995) was purified using PC-Sepharose affinity chromatography (Skerra & Pluckthun, 1988) in the presence or absence of co-expressed Skp. The concentration and yield was estimated photometrically using a calculated extinction coefficient (Gill & von Hippel, 1989).

Detail Description Paragraph:
[0108] We then determined the effect of Skp on the production of several of the scFv fragments in soluble form using the non-suppressor strain JM83. Using antigen-binding ELISA (FIG. 6) it can be seen that the amount of soluble scFv was dramatically increased in the presence of co-expressed Skp. To demonstrate that this is also reflected in the yield of purified protein, the scFv fragment of the anti-phosphorylcholine binding antibody McPC603-H11 (Knappik & Pluckthun, 1995) was tested. Co-expression of Skp increased the amount of protein, purified by affinity-chromatography on phosphorylcholine by about a factor of 4.

L7: Entry 7 of 30 File: PGPB Mar 18, 2004

DOCUMENT-IDENTIFIER: US 20040052779 A1
TITLE: Opsonic monoclonal and chimeric antibodies specific for lipoteichoic acid of Gram positive bacteria

Abstract Paragraph:
The present invention encompasses monoclonal antibodies that bind to lipoteichoic acid (LTA) of Gram positive bacteria. The antibodies also bind to whole bacteria and enhance phagocytosis and killing of the bacteria in vitro. The invention also provides antibodies having human sequences (chimeric, humanized and human antibodies). The invention also sets forth the variable regions of three antibodies within the invention and presents the striking homology between them.

Summary of Invention Paragraph:
[0010] Further exacerbating the problem, the role of the common surface antigens on staphylococci has been unclear. For example, while lipoteichoic acid and teichoic acid make up the majority of the cell wall of S. aureus, there was no prior appreciation that antibodies to lipoteichoic acid and teichoic acid could be protective. Indeed, anti-teichoic acid antibodies have been often used as controls. For example, Fattom et al. examined the opsonic activity of antibodies induced against a type-specific capsular polysaccharide of S. epidermidis, using as controls antibodies induced against teichoic acids and against S. hominus. While type-specific antibodies were highly opsonic, anti-teichoic acid antibodies were not functionally different from the anti-S. hominus antibodies (6).

Detail Description Paragraph:
[0200] 3. Endl, J.; Seidl, H. P.; Fiedler, F.; and Schleifer, K. H. 1983. Chemical composition and structure of cell wall teichoic acid of staphylococci, Arch Microbiol, 135: 215-223.

CLAIMS:

1. A MAb comprising at least one light chain and at least one heavy chain, wherein said at least one light chain comprises a polypeptide comprising an amino acid sequence having at least 70% identity with a light chain variable region selected from Seq. ID Nos. 16, 10, and 21; wherein said at least one heavy chain comprises a polypeptide comprising an amino acid sequence having at least 70% identity with a heavy chain variable region selected from Seq. ID Nos. 12,17, or 22; and wherein said MAb specifically binds to LTA.

2. The Mab according to claim 1, wherein the percents identity are at least 80%.

3. The Mab according to claim 1, wherein the percents identity are at least 90%.

4. The MAb of claim 1, comprising at least one variable region having an amino acid sequence selected from Seq. ID Nos. 10, 12, 16, 17, 21, and 22.

5. The MAb according to claim 1, wherein at least one light chain, at least one heavy chain, or both are chimeric or humanized.

6. The MAb according to claim 1, wherein at least one light chain, at least one

DOCUMENT-IDENTIFIER: US 4150116 A
TITLE: Immunization against dental caries with glucosyltransferase antigens

Detailed Description Text (62):
Previously we have suggested that two likely antigens of S. mutans for immunization experiments might be either the serotype-specific carbohydrate antigen or the GTF enzymes. Smith, D. J., and M. S. Taubman. 1976. Immunization experiments using the rodent caries model. J. Dent Res. 55 (Special Issue C):C193. This suggestion was based on the demonstration that antibody directed to either of these antigens had the capacity to interfere with adherence phenomena demonstrated by S. mutans in vitro. Mukasa, H., and H. D. Slade. 1974. Mechanism of adherence of Streptococcus mutans to smooth surfaces. II. Nature of the binding site and the adsorption of dextran-levan synthetase enzyme on the cell wall surface of the streptococcus. Infect. Immun. 9:419, Iacono, V. J., M. A. Taubman, D. J. Smith, P. R. Garant, and J. R. Pollock. 1976. Structure and function of the type-specific polysaccharide of Streptococcus mutans 6715. Immunology Abstracts (Special Suppl.):75. Although both antigens occur in culture supernatants, in the current invention, we have been careful to eliminate type-specific antigen from all our GTF preparations used for immunization. Antibody reactive with the type-specific antigen was never detected in the serums of any of the animals immunized with these enzyme preparations. The levels of protection reported in the above examples, utilizing CE-1 and CE-2 GTF as an antigen in the rat models, are quite comparable to the levels of protection obtained previously after immunization with whole cells. Taubman, Smith, supra. The experiment in hamsters with more defined GTF enzymes (DE-1 and DE-2) as immunogens also supports the contention that GTF enzyme is of major importance as antigen. Although the evidence is not unequivocal, there are several additional compelling reasons in support of the case for GTF enzyme: (a) Other enzyme antigens (e.g. fructosyltransferase or invertase) were probably absent from our GTF fractions. (b) Both GTF enzyme preparations, having only one enzyme antigen in common, gave rise to protection. (c) Although the DE-1 preparation contained trace amounts of material reactive with an antiserum directed to the polyglycerol phosphate (PGP) backbone of teichoic acid, as did DE-2, serum antibody from hamsters immunized with either of these preparations did not react with teichoic acid from S. Sanguis. (d) Immunized animals showed antibody in serum and saliva which would bind and inhibit GTF activity. (3) The likelihood of DE-1 and DE-2 containing common antigens other than enzyme is low due to the complex series of procedure followed and the purposeful selection for material demonstrating enzyme (GTF) activity. Nevertheless, it is clear that even more purified enzyme as antigen would better establish the importance of GTF in the pathogenesis of S. mutans and also the importance of GTF as antigen for immunization.

heavy chain, or both, are human.

7. The MAb according to claim 1, comprising a heavy chain constant region; wherein said constant region comprises human IgG, IgA, IgM, or IgD sequence.

8. The MAb of claim 1, comprising a Fab, Fab', F(ab').sub.2, Fv, SFv, scFv.

9. A polypeptide comprising an amino acid sequence having at least 70% identity with a light chain variable region selected from Seq. ID Nos. 16, 10, and 21; wherein said polypeptide is capable of functioning as a variable region, or portion thereof, in a MAb that specifically binds to LTA.

10. The polypeptide according to claim 9, comprising at least one region having at least 88% identity with a sequence selected from amino acids 24-33, 49-55, and 88-73 of Seq. ID Nos. 10,16, or 21; wherein said region is capable of functioning as a CDR, or portion thereof, in a MAb that specifically binds to LTA.

11. The polypeptide according to claim 9, comprising at least one region having at least 82% identity with a sequence selected from amino acids amino acids 1-23, 34-38, 56-87, and 97-106 of Seq. ID Nos. 10, 16, or 21; wherein said region is capable of functioning as a framework region, or portion thereof, in a MAb that specifically binds to LTA.

12. A MAb light chain comprising the polypeptide according to claim 9.

13. The Mab light chain according to claim 12, wherein said light chain is chimeric, humanized, or human.

14. The MAb light chain according to claim 12, comprising a light chain constant region comprising human kappa or lambda sequence.

15. A polypeptide comprising an amino acid sequence having at least 70% identity with a heavy chain variable region selected from Seq. ID Nos. 12, 17, or 22; wherein said polypeptide is capable of functioning as a variable region, or portion thereof, in a MAb that specifically binds to LTA.

16. The polypeptide according to claim 15, comprising at least one region having at least 80% identity with a sequence selected from amino acids 26-35, and 50-69 of Seq. ID Nos. 12,17, or 22; wherein said region is capable of functioning as a CDR, or portion thereof, in a MAb that specifically binds to LTA.

17. The polypeptide according to claim 15, comprising at least one region having at least 80% identity with a sequence selected from amino acids amino acids 1-25, 36-49, 70-101, and 115-125 Seq. ID Nos. 12,17, or 22; wherein said region is capable of functioning as a framework region, or portion thereof, in a MAb that specifically binds to LTA.

18. A MAb heavy chain comprising the polypeptide according to claim 15.

19. The Mab heavy chain according to claim 18, wherein said heavy chain is chimeric, humanized, or human.

20. The MAb heavy chain according to claim 18, comprising a heavy chain constant region comprising human IgG, IgA, IgM, or IgD sequence.

21. A MAb comprising at least one light chain and at least one heavy chain, wherein said MAb specifically binds LTA; and wherein said at least one light chain comprises a variable region having at least one CDR comprising a sequence selected from amino acids 24-33, 49-55, or 88-73 of Seq. ID Nos. 10, 16, or 21; or wherein said at least one light chain comprises a variable region having at least one CDR

comprising a sequence selected from amino acids 1-25, 36-49, 70-101, or 115-125 of Seq. ID Nos. 12,17, or 22.

22. A Mab according to claim 21, comprising at least one variable domain selected from A110, A110b, A120, A120b, and 391.4.

23. A hybridoma cell line expressing a MAb according to claim 22.

24. A pharmaceutical composition comprising one or more Mabs according to claim 1 and a pharmaceutically acceptable carrier.

31. A method of making the MAb of claim 1 comprising the steps of: a) selecting at least one Mab that specifically binds to at least of LTA, or a peptide mimeotope of LTA that induces anti-LTA antibodies; b) determining the polypeptide sequence of the light chain variable region of said at least one Mab; c) selecting a polypeptide sequence having at least 70% identity with a light chain variable region selected from Seq. ID Nos. 16,10, and 21; d) determining the polypeptide sequence of the heavy chain variable region of said at least one Mab; e) selecting a polypeptide sequence having at least 70% identity with a heavy chain variable region selected from Seq. ID Nos. 12,17, or 22; f) combining a light chain comprising a polypeptide sequence of step c) with a heavy chain comprising a polypeptide sequence of step e).

32. A method of making the polypeptide of claim 9, comprising the steps of: a) selecting at least one Mab that specifically binds to at least of LTA, or a peptide mimeotope of LTA that induces anti-LTA antibodies; b) determining the polypeptide sequence of the light chain variable region of said at least one Mab; d) selecting a polypeptide sequence having at least 70% identity with a light chain variable region selected from Seq. ID Nos. 16, 10, and 21.

33. A method of making the polypeptide of claim 15, comprising the steps of: a) selecting at least one Mab that specifically binds to at least of LTA, or a peptide mimeotope of LTA that induces anti-LTA antibodies; b) determining the polypeptide sequence of the heavy chain variable region of said at least one Mab; d) selecting a polypeptide sequence having at least 70% identity with a heavy chain variable region selected from Seq. ID Nos. 12, 17, or 22.

36. A production system comprising, 1) a cell; and 2) one or more recombinant nucleic acids capable of directing the expression of a Mab according to claim 1.

37. A method of identifying highly antigenic and highly conserved epitopes comprising the steps of: a) selecting a multiplicity of MAbs that specifically binds to an immunogen; b) determining the polypeptide sequence of the variable regions of said MAbs; d) identifying regions of identity in the polypeptide sequence of at least two of said Mabs, said regions of identity comprising at least one of 1) at least 70% identity of light chain variable regions, at least 70% identity of heavy chain variable regions, at least 70% identity over 3 complementarity determining regions (CDRs) in a variable region, at least 75% identity over at least two CDRs in a variable region; at least 80% identity in a CDR; and at least 70% identity in the framework regions (FRs) of a variable region.

38. A collection of Mabs that bind to LTA comprising, a multiplicity of Mabs according to claim 1.

39. The collection of claim 38, wherein the collection comprises one or more of M110, M120, 391.4, or a chimeric or humanized derivative thereof.

Previous Doc Next Doc Go to Doc#

Detail Description Paragraph:
[0190] 3. Endl, J.; Seidl, H. P.; Fiedler, F.; and Schleifer, K. H. 1983. Chemical composition and structure of cell wall teichoic acid of staphylococci, Arch Microbiol, 135: 215-223.

L12: Entry 7 of 28 File: USPT Aug 17, 2004

DOCUMENT-IDENTIFIER: US 6777193 B1
TITLE: Methods for diagnostic and/or treatment of antiphospholipids antibodies-related diseases, and devices

Drawing Description Text (15):
FIG. 14 illustrates the graphs of the inhibition of H308 monoclonal antibody with phosphorylcholine, glycerolphosphorylcholine, phosphorylserine, glycerolphosphorylserine or phosphorylethanolamine haptens. Furthermore, the reaction of H308 monoclonal antibody with liposomes made from phosphorylcholine is also showed.

Detailed Description Text (89):
An additional aspect of the present invention, consists of preventing or treating illnesses associated with antiphospholipid antibodies by means of the administration of a therapeutically effective quantity of a drug for inhibition or blocking of the anti-lipidic particles antibodies from sick persons, or, by means of the administration of a therapeutically effective quantity of a stabilizer drug to achieve the stabilization of cellular membranes from sick persons. The above-mentioned processes are achieved in vitro by means of inhibition or blocking of the anti-lipidic particles antibodies from sick persons with phosphorylated haptens, which are chemical substances that are part of the polar region of the cellular membrane lipids; in a similar way as it has been demonstrated in the inhibition of H308 monoclonal antibody by phosphorylcholine and glycerolphosphoryl-choline haptens (Aguilar, op. cit. 1997).

Detailed Description Text (276):
Inhibition of H308 monoclonal antibody reaction with phosphorylcholine and glycerolphosphorylcholine indicate that the antigen recognition domain in H308 monoclonal antibody has subdomains that recognize specifically the choline methyl groups which lacks ethanolamine and serine (FIG. 13). In addition, total immunoreaction inhibition attained by glycerolphosphorylcholine suggests that the antigen domain that recognize H308 monoclonal antibody include chemical groups of glycerol. These findings are in agreement with the structural pattern proposed for the lipidic particle (Cullis et al., op. cit., 1991) (FIG. 15) where monolayer lipids (C, FIG. 15) that recover the molecular arrangement different to bilayer (B, FIG. 15) are more separate than lipids that constitute a normal monolayer (A, FIG. 15). In an open monolayer (C, FIG. 15) glycerolphosphorylcholine is more exposed than in a normal bilayer therefore this is the region in which the H308 monoclonal antibody reacts.

DOCUMENT-IDENTIFIER: US 5665347 A
TITLE: IL-12 inhibition of B1 cell activity

Drawing Description Text (2):
FIGS. 1A-1D are graphs of the reciprocal serum dilution versus optical density (OD) at 405 nm demonstrating suppression of the IgG1 and enhancement of the IgG2a anti-phosphorylcholine (anti-PC) response after IL-12 treatment (solid lines indicate mice receiving IL-12 and dashed lines indicate mice receiving phosphate buffered saline (PBS)).

Previous Doc Next Doc Go to Doc#
First Hit Fwd Refs

Generate Collection

L10: Entry 39 of 43 File: USPT Aug 6, 1996

DOCUMENT-IDENTIFIER: US 5543144 A
TITLE: Treating hypersensitivities with anti-IGE monoclonal antibodies which bind to IGE-expressing B cells but not basophils

Detailed Description Text (103):
RA25 rats were immunized with a mixture of purified murine monoclonal antibodies of immunoglobulin class IgE (IgE MAbs) composed of three different anti-phosphorylcholine (PC) IgE (designated aPC4-33, aPC12-3 and aPC71-130) as well as an anti-dinitrophenol IgE (designated aDNP 69-3).

Previous Doc Next Doc Go to Doc#

Generate Collection | Print

**Search Results** - Record(s) 1 through 43 of 43 returned.

☐ 1. 20040185039. 29 Aug 03. 23 Sep 04. Therapeutic applications of noncovalent dimerizing antibodies. Kohler, Heinz, et al. 424/131.1; A61K039/395.

☐ 2. 20040152103. 19 Aug 03. 05 Aug 04. Novel methods for obtaining, identifying and applying nucleic acid sequences and (poly)peptides which increase the expression yields of periplasmic proteins in functional form. Plueckthun, Andreas, et al. 435/6; 435/456 702/20 C12Q001/68 G06F019/00 G01N033/48 G01N033/50 C12N015/86.

☐ 3. 20040077847. 19 Nov 02. 22 Apr 04. Pneumococcal genes, portions thereof, expression products therefrom, and uses of such genes, portions and products. Briles, David E., et al. 536/23.1; C07H021/02 C07H021/04.

☐ 4. 20030166876. 12 Jun 02. 04 Sep 03. Method of treating cancer including administering a human chimeric antibody specific for the ganglioside GD3. Shitara, Kenya, et al. 530/388.85; 424/155.1 435/320.1 435/70.21 A61K039/395 C12P021/04 C12N005/06 C07K016/30.

☐ 5. 20030148484. 16 Jan 03. 07 Aug 03. Catalytic antibodies and a method of producing same. Koentgen, Frank, et al. 435/188.5; 435/320.1 435/326 435/68.1 435/69.1 530/399 536/23.53 C12N009/00 C07H021/04 C12P021/06 C07K014/475 C07K016/22 C12N005/06.

☐ 6. 20030095964. 08 Oct 02. 22 May 03. Process for producing humanized chimera antibody. Shitara, Kenya, et al. 424/131.1; 435/320.1 435/327 435/69.1 530/387.3 536/23.53 A61K039/395 C07H021/04 C12P021/02 C07K016/44 C12N005/06.

☐ 7. 20030077276. 16 Jul 02. 24 Apr 03. Humanized antibodies. Nakamura, Kazuyasu, et al. 424/141.1; 530/388.15 536/53 A61K039/395 C08B037/00 C07K016/44.

☐ 8. 20020028200. 10 May 01. 07 Mar 02. Anti-idiotypic antibody against FimH adhesion of uropathogenic type I-fimbriated escherichia coli, compositions containing same and method for using same. Wu, Xue-Ru. 424/131.1; 435/327 530/387.2 A61K039/395 C12N005/06 C07K016/42.

☐ 9. 20020026036. 19 Jan 01. 28 Feb 02. Process for producing humanized chimera antibody. Shitara, Kenya, et al. 530/388.85; 424/155.1 435/69.1 C12P021/02 A61K039/395 C07K016/30.

☐ 10. 6916475. 15 Oct 02; 12 Jul 05. Anti-idiotypic antibody and its use in regulating the composition of T cell lymphocytes. Muller; Sybille, et al. 424/131.1; 424/141.1 424/154.1 530/387.2 530/388.1. A61K039/395.

☐ 11. 6872392. 16 Jul 02; 29 Mar 05. Humanized antibodies. Nakamura; Kazuyasu, et al. 424/133.1; 424/137.1 424/141.1 530/388.1. A61K039/395 A61K039/40 A61K039/42 C07K016/00.

☐ 12. 6632926. 16 Nov 99; 14 Oct 03. Antibody variants. Chen; Yvonne M., et al. 530/387.3; 530/388.85. C07K016/00.

☐ 13. 6632431. 10 May 01; 14 Oct 03. Anti-idiotypic antibody against FimH adhesion of uropathogenic type I-fimbriated Escherichia coli, compositions containing same and method for using

same. Wu; Xue-Ru. 424/131.1; 424/141.1 424/184.1 424/204.1 424/206.1 424/239.1 424/259.1 424/274.1 424/422 424/423 424/426 435/7.1 530/387.1 530/387.2. A61K039/395.

---

☐ 14. 6630317. 01 May 00; 07 Oct 03. Methods for obtaining, identifying and applying nucleic acid sequences and (poly)peptides which increase the expression yields of periplasmic proteins in functional form. Pluckthun; Andreas, et al. 435/7.4; 435/7.1 435/DIG.15 435/DIG.17 435/DIG.8. G01N033/573 G01N033/53.

---

☐ 15. 6590080. 09 Jun 00; 08 Jul 03. Catalytic antibodies and a method of producing same. Koentgen; Frank, et al. 530/399; 435/188.5. C07K014/60 C12N009/00.

---

☐ 16. 6585973. 28 Oct 99; 01 Jul 03. Method for preparing solid phase conjugated vaccine. Lees; Andrew. 424/193.1; 424/194.1 424/196.11 424/197.11. A61K039/385.

---

☐ 17. 6521741. 09 Nov 00; 18 Feb 03. Catalytic antibodies and a method of producing same. Koentgen; Frank, et al. 530/350; 435/188.5. C07K014/00 C07K002/00 C12N009/00.

---

☐ 18. 6500613. 16 Sep 96; 31 Dec 02. Pneumococcal surface proteins and uses thereof. Briles; David E., et al. 435/6; 424/184.1 424/244.1 536/23.7. C12Q001/68 A61K039/00 A61K039/09 C07H021/04.

---

☐ 19. 6495666. 19 Jan 01; 17 Dec 02. Polypeptide composing human chimeric antibody. Shitara; Kenya, et al. 530/350; 530/387.1 530/388.8 530/388.85. C07K014/00.

---

☐ 20. 6465173. 25 Jul 01; 15 Oct 02. Anti-idiotypic antibody and its use in diagnosis and therapy in HIV-related disease. Muller; Sybille, et al. 435/5; 435/339 435/339.1 435/344.1 435/7.1 435/7.9 435/974 436/518 436/536 530/387.2. C12Q001/70.

---

☐ 21. 6461612. 28 Feb 01; 08 Oct 02. Anti-idiotypic antibody and its use in diagnosis and therapy in HIV-related disease. Muller; Sybille, et al. 424/131.1; 424/143.1 424/154.1 530/387.2. A61K039/395.

---

☐ 22. 6437098. 05 Jan 99; 20 Aug 02. Human chimeric antibody specific for the ganglioside GD3. Shitara; Kenya, et al. 530/388.85; 424/130.1 424/133.1 530/387.1 530/387.3 530/388.8. C07K016/30.

---

☐ 23. 6423511. 10 Sep 99; 23 Jul 02. Humanized antibodies. Nakamura; Kazuyasu, et al. 435/69.6; 435/252.3 435/320.1 435/325 435/328 435/71.1 536/23.53. C12P021/04 C12N001/20 C12N015/00 C12N005/00 C07H021/04.

---

☐ 24. 6326179. 25 Sep 98; 04 Dec 01. Catalytic antibodies and a method of producing same. Koentgen; Frank, et al. 435/188.5; 424/175.1 424/94.1. C12N009/60 A61K038/47.

---

☐ 25. 6221580. 05 Jul 00; 24 Apr 01. Anti-idiotypic and its uses in diagnosis and therapy in HIV-related disease. Muller; Sybille, et al. 435/5; 435/339 435/339.1 435/344.1 435/7.1 435/7.9 435/974 436/518 436/536 530/387.2. C12Q001/70.

---

☐ 26. 6217866. 07 Jun 95; 17 Apr 01. Monoclonal antibodies specific to human epidermal growth factor receptor and therapeutic methods employing same. Schlessinger; Joseph, et al. 424/143.1; 424/130.1 424/138.1 424/141.1 424/152.1 424/155.1 424/156.1 530/388.1 530/388.2 530/388.22 530/388.8 530/388.85. A61K039/395 C07K016/28.

---

☐ 27. 6146627. 14 Dec 98; 14 Nov 00. Method for reducing T cell-mediated cytotoxicity in HIV using anti-idiotypic antibody. Muller; Sybille, et al. 424/131.1; 424/133.1 424/141.1 530/387.2.

C07K016/00.

---

☐ 28. 6057421. 03 Dec 97; 02 May 00. Variable heavy and light chain regions of murine monoclonal antibody 1F7. Muller; Sybille, et al. 530/300; 424/131.1 424/138.1 424/174.1 424/178.1 435/327 530/387.2 530/387.7 530/388.8 530/389.7 530/391.3. A61K039/395 C07K007/04.

---

☐ 29. 6043069. 26 Mar 97; 28 Mar 00. Catalytic antibodies and a method of producing same. Koentgen; Frank, et al. 435/188.5;. C12N009/00.

---

☐ 30. 6042828. 27 Jun 96; 28 Mar 00. Humanized antibodies to ganglioside GM.sub.2. Nakamura; Kazuyasu, et al. 424/133.1; 424/137.1 424/141.1 435/320.1 435/326 435/328 435/329 435/69.6 435/7.23 435/70.21 530/387.3 530/387.5 530/867 536/23.53. A61K039/395 A61K039/40 C12P021/04 C12P021/08.

---

☐ 31. 5939532. 07 Jun 95; 17 Aug 99. Humanized antibodies to ganglioside GM.sub.2. Nakamura; Kazuyasu, et al. 530/387.3; 424/141.1 435/328 435/329 435/69.3 530/387.5 530/388.1. C12P021/08 C12P021/06 C07K016/00 C12N005/00.

---

☐ 32. 5874255. 10 May 95; 23 Feb 99. DNA encoding humanized antibodies specific for the ganglioside GM.sub.2. Nakamura; Kazuyasu, et al. 435/69.9; 435/320.1 435/328 435/330 435/332 435/344.1 435/346 435/70.21 530/387.3 530/387.5 530/387.7 530/388.1 530/388.2 530/867 536/23.53. C12P021/04 C12P021/08 C12N015/00 C07H021/04.

---

☐ 33. 5866692. 31 May 95; 02 Feb 99. Process for producing humanized chimera antibody. Shitara; Kenya, et al. 536/23.1; 536/24.2. C12N015/11.

---

☐ 34. 5849583. 20 Aug 93; 15 Dec 98. Anti-idiotypic antibody and its use in diagnosis and therapy in HIV-related disease. Muller; Sybille, et al. 435/346; 435/327 530/387.1 530/387.2 530/388.1 530/866. C07K016/42 C12N005/20.

---

☐ 35. 5830470. 07 Sep 93; 03 Nov 98. Humanized antibodies to ganglioside GM.sub.2. Nakamura; Kazuyasu, et al. 424/133.1; 424/137.1 435/320.1 435/328 435/330 435/69.6 435/70.21 530/387.3 530/387.5 530/388.8 536/23.53. A61K039/395 C12P021/04 C12P021/08 C07H021/04.

---

☐ 36. 5807548. 31 May 95; 15 Sep 98. Method of treating cancer using a chimera antibody. Shitara; Kenya, et al. 424/133.1; 424/130.1 424/137.1. A61K039/395 C07K016/46.

---

☐ 37. 5750078. 21 Mar 95; 12 May 98. Process for producing humanized chimera antibody. Shitara; Kenya, et al. 424/133.1; 424/130.1 424/137.1 435/252.3 435/328 435/69.6 435/70.21 530/387.3 530/387.5 530/388.8 530/388.85 536/23.53. C07K016/46 A61K039/395.

---

☐ 38. 5665347. 02 Feb 95; 09 Sep 97. IL-12 inhibition of B1 cell activity. Metzger; Dennis W., et al. 424/85.2; 514/12 514/21. A61K038/20 A61K045/05.

---

☐ 39. 5543144. 21 Jan 93; 06 Aug 96. Treating hypersensitivities with anti-IGE monoclonal antibodies which bind to IGE-expressing B cells but not basophils. Chang; Tse W.. 424/133.1; 424/139.1 424/145.1 530/387.3 530/388.25. A61K039/395 C07K016/42.

---

☐ 40. 5475100. 19 Aug 93; 12 Dec 95. Artificial antibody. Hashino; Kimikazu, et al. 536/23.53; 530/387.3 536/23.1 536/23.5. C07H021/00 C07K016/00.

---

☐ 41. WO2004091520A2. 12 Apr 04. 28 Oct 04. METHODS AND COMPOSITIONS FOR TREATING ATHEROSCLEROSIS. WITZTUM, JOSEPH L, et al. A61K00/;.

---

☐ 42. WO009012632A1. 06 Apr 90. 01 Nov 90. PROCESS FOR REMOVING C-REACTIVE PROTEIN AND ANTI-PHOSPHORYLCHOLINE ANTIBODIES FROM BIOLOGICAL FLUIDS. KEDAR, IGAL. B01D015/08;.

---

☐ 43. CN 1536365A. Quantitative determination of oxidative low-density lipoprotein by using anti-phosphorylcholine antibody and its application in diagnosis of atherosis. XIAO, X. A61B005/00 G01N033/53 G01N033/577 G01N033/68 G01N033/92.

---

Generate Collection Print

| Term | Documents |
|---|---|
| (9 NOT 8).PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD. | 43 |
| (L9 NOT L8).PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD. | 43 |

Prev Page Next Page Go to Doc#

# WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Friday, September 16, 2005

| Hide? | Set Name | Query | Hit Count |
|---|---|---|---|
| | | *DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR* | |
| ☐ | L1 | teicho$ near acid$ | 326 |
| ☐ | L2 | L1 and wall | 239 |
| ☐ | L3 | L2 and (polyclonal or poly-clonal or antisera or antiserum or anti-sera or anti-serum or immunoglobulin or globulin or igg or igm or iga or ig or monoclonal or hybridoma or mono-clonal or mab or moab or m-ab or chimeric or humanized or ivig or igiv or iv-ig ivigg) | 177 |
| ☐ | L4 | L1 same wall | 147 |
| ☐ | L5 | L4 and l3 | 103 |
| ☐ | L6 | L4 same (polyclonal or poly-clonal or antisera or antiserum or anti-sera or anti-serum or immunoglobulin or globulin or igg or igm or iga or ig or monoclonal or hybridoma or mono-clonal or mab or moab or m-ab or chimeric or humanized or ivig or igiv or iv-ig ivigg) | 11 |
| ☐ | L7 | L4 and (polyclonal or poly-clonal or antisera or antiserum or anti-sera or anti-serum or immunoglobulin or globulin or igg or igm or iga or ig or monoclonal or hybridoma or mono-clonal or mab or moab or m-ab or chimeric or humanized or ivig or igiv or iv-ig ivigg).ti,ab,clm. not l6 | 30 |
| ☐ | L8 | antiphosphorylcholine | 4 |
| ☐ | L9 | anti-phosphorylcholine | 47 |
| ☐ | L10 | L9 not l8 | 43 |
| ☐ | L11 | L9 not l8 | 43 |
| ☐ | L12 | antibod$ near2 phosphorylcholine not l10 not l11 not l8 not l6 | 28 |

END OF SEARCH HISTORY

Generate Collection | Print

**Search Results** - Record(s) 1 through 11 of 11 returned.

☐ 1. 20050158346. 18 Jan 05. 21 Jul 05. Antimultiorganism Glycoconjugate vaccine. Kubler-Kielb, Joanna, et al. 424/246.1; 530/395 536/54 A61K039/00 A61K039/38 C07K014/32.

☐ 2. 20040247605. 01 Dec 03. 09 Dec 04. Wall teichoic acid as a target for anti-staphylococcal therapies and vaccines. Kokai-Kun, John Fitzgerald, et al. 424/184.1; A61K039/00 A61K039/38.

☐ 3. 20040228879. 15 Jun 04. 18 Nov 04. Combination vaccine against streptococcus pneumoniae and respiratory syncytial virus (RSV). Deschamps, Marguerite, et al. 424/211.1; A61K039/155.

☐ 4. 20040120966. 05 Jan 04. 24 Jun 04. Polypeptide comprising the amino acid of an N-terminal choline binding protein a truncate, vaccine derived therefrom and uses thereof. Tuomanen, Elaine I., et al. 424/184.1; A61K039/00 A61K039/38.

☐ 5. 20030228322. 20 Dec 02. 11 Dec 03. Multifunctional monoclonal antibodies directed to peptidoglycan of gram-positive bacteria. Schuman, Richard F., et al. 424/184.1; A61K039/00 A61K039/38.

☐ 6. 20030096950. 07 Apr 98. 22 May 03. A POLYPEPTIDE COMPRISING THE AMINO ACID OF AN N-TERMINAL CHOLINE BINDING PROTEIN A TRUNCATE, VACCINE DERIVED THEREFROM AND USES THEREOF. TUOMANEN, ELAINE I., et al. 530/350; 424/184.1 530/300 C07K001/00 A61K038/00 A61K039/00.

☐ 7. 6858706. 07 Apr 98; 22 Feb 05. Polypeptide comprising the amino acid of an N-terminal choline binding protein a truncate, vaccine derived therefrom and uses thereof. Tuomanen; Elaine I., et al. 530/350; 424/184.1 424/185.1 424/190.1 424/234.1 424/244.1 530/324. A61K038/00 A61K039/09 A61K039/38 A61K039/02 A61K039/00 C07K016/00 C07K017/00 C07K007/00 C07K005/00 C07K001/00.

☐ 8. 5290707. 25 Nov 91; 01 Mar 94. Method for detection of microorganisms. Wood; Sheila J.. 436/523; 435/7.93 435/973 436/533 436/534 436/536 436/538 436/541 436/800 436/824. G01N033/544 G01N033/545 G01N033/546.

☐ 9. 5139933. 25 Jun 90; 18 Aug 92. Assay method for detecting listeria. Green; Calvert L., et al. 435/7.32; 435/176 435/29 435/7.92 435/820 435/961 436/526 436/532. G01N033/569.

☐ 10. 4250262. 14 Dec 79; 10 Feb 81. Method of preparing a purified glucosyltransferase. Taubman; Martin A., et al. 435/193; 435/815 435/885. C12N009/10.

☐ 11. 4150116. 21 Feb 78; 17 Apr 79. Immunization against dental caries with glucosyltransferase antigens. Taubman; Martin A., et al. 424/244.1; 424/50 424/94.5 435/193. A61K039/00 A61K037/48 A61K007/28.

Generate Collection | Print

| Term | Documents |
|---|---|
| | |

| | |
|---|---|
| POLYCLONAL | 56968 |
| POLYCLONALS | 291 |
| POLY-CLONAL | 331 |
| POLY-CLONALS | 0 |
| ANTISERA | 23612 |
| ANTISERAS | 18 |
| ANTISERUM | 21085 |
| ANTISERUMS | 228 |
| ANTI-SERA | 1229 |
| ANTI-SERAS | 1 |
| ANTI-SERUM | 1990 |
| (L4 SAME (POLYCLONAL OR POLY-CLONAL OR ANTISERA OR ANTISERUM OR ANTI-SERA OR ANTI-SERUM OR IMMUNOGLOBULIN OR GLOBULIN OR IGG OR IGM OR IGA OR IG OR MONOCLONAL OR HYBRIDOMA OR MONO-CLONAL OR MAB OR MOAB OR M-AB OR CHIMERIC OR HUMANIZED OR IVIG OR IGIV OR IV-IG IVIGG)).PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD. | 11 |

There are more results than shown above. Click here to view the entire set.

Prev Page Next Page Go to Doc#

L7: Entry 14 of 30 File: PGPB Jun 5, 2003

PGPUB-DOCUMENT-NUMBER: 20030103969
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030103969 A1

TITLE: Use of antibodies to block the effects of Gram-positive bacteria and mycobacteria

PUBLICATION-DATE: June 5, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY |
|---|---|---|---|
| Ulevitch, Richard J. | Del Mar | CA | US |
| Tobias, Peter S. | San Diego | CA | US |
| Pugin, Jerome | Puplinge | | CH |

US-CL-CURRENT: 424/140.1

CLAIMS:

1. A method of ameliorating one or more symptoms of Gram positive bacterial or mycobacterial sepsis in a subject comprising administering to the subject a therapeutically effective amount of an antibody having the same immunoreactivity as an antibody produced by ATCC Accession No. HB11364 (28C5) or an immunoreactive fragment thereof.

2. The method of claim 1, wherein the antibody is the antibody produced by ATCC Accession No. HB11364 (28C5).

3. The method of claim 1, wherein the antibody is comprised of F(ab').sub.2 portions.

4. The method of claim 1, wherein the therapeutically effective amount is 0.1 to 20 milligrams per kilogram body weight per day.

5. The method of claim 1, wherein the method further comprises administering to said patient a bactericidal amount of an antibiotic.

6. The method of claim 5, wherein said antibiotic is an anti-bacterial agent effective against Gram-positive bacteria.

7. The method of claim 1 wherein the method further comprises administering to the subject a TNF blood concentration-reducing amount of an anti-TNF antibody.

8. The method of claim 1 wherein the subject displays symptoms of one or more of the following: adult respiratory distress syndrome, disseminated intravascular coagulation, renal failure and hepatic failure.

9. A method of ameliorating Gram positive bacterial or my cobacterial sepsis in a subject comprising administering to the subject a therapeutically effective amount of an antibody having the same immunoreactivity as an antibody produced by ATCC Accession No. HB44 (63D3) or an immunoreactive fragment thereof.

10. The method of claim 9, wherein the antibody is the antibody produced by ATCC Accession No. HB44 (63D3).

11. The method of claim 9, wherein said monoclonal antibody is comprised of F (ab').sub.2 portions of anti-CD14 antibody molecules.

12. The method of claim 9 wherein said therapeutically effective amount is 0.1 to 20 milligrams per kilogram body weight per day.

13. The method of claim 9 wherein said method further comprises substantially simultaneously administering to said patient a bactericidal amount of an antibiotic.

14. The method of claim 13 wherein said antibiotic is an anti-bacterial agent effective against Gram-positive bacteria.

15. The method of claim 9 wherein the method further comprises administering to the subject patient a TNF blood concentration-reducing amount of an anti-TNF antibody.

16. The method of claim 9 wherein the subject displays symptoms of one or more of the following: adult respiratory distress syndrome, disseminated intravascular coagulation, renal failure and hepatic failure.

17. A method of ameliorating one or more symptoms of Gram positive bacterial or mycobacterial sepsis in a subject comprising administering to the subject, a therapeutically effective amount of an antibody that inhibits binding of Gram-positive toxigenic cell wall components to CD14, and inhibits secretion of tumor necrosis factor by cells of the monocyte macrophage lineage, thereby treating the sepsis.

18. The method of claim 17 wherein the antibody is a monoclonal antibody that competitively inhibits the binding of the toxigenic cell wall components to CD14.

19. The method of claim 17 wherein the antibody is produced by hybridoma ATCC HB44 or ATCC HB11364 or a host cell containing a polynucleotide encoding a 63D3 or 28C5 antibody.

20. The method of claim 17 wherein the Gram-positive bacteria is Staphylococcus aureus.

21. The method of claim 17 wherein the Gram-positive bacteria is selected from the group consisting of Group A and Group B Streptococci.

22. The method of claim 17 wherein the Gram-positive bacteria is Streptococcus pneumoniae.

23. The method of claim 17 wherein the mycobacterium is Mycobacterium tuberculosis.

Previous Doc Next Doc Go to Doc#

Detail Description Paragraph:
[0550] Nordenstam G, B. Anderson, D. E. Briles, J. Brooks, A. Oden, A. Svanborg and C. S. Eden. 1990. High anti-phosphorylcholine antibody levels and mortality associated with pneumonia. Scand. J. Infect. Dis. 22:187.

Detail Description Paragraph:
[0143] Using a mRNA extraction kit, Fast Track (No. K1593-02, available from Invitrogen), 6.2 .mu.g of mRNA was obtained from 1.times.10.sup.8 cells of chimera antibody-producing SP2-PC Chimera-1 which has anti-phosphorylcholine activity and is disclosed in FEBS Letters (244, 301-306 (1989)).

DOCUMENT-IDENTIFIER: US 20030148484 A1
TITLE: Catalytic antibodies and a method of producing same

Detail Description Paragraph:
[0208] In order to allow for purification of correctly folded growth factor precursors, the blocking entity was therefore redesigned. Kappa will be replaced by a single chain (sc) antibody which is stabilised by an internal disulphide bridge (disulphide bridge stabilised, ds). This scdsFv will be derived from the extensively described plasmacytoma McPc603 [Freund et al. Biochemistry 33: 3296-3303, 1994] with anti-phosphorylcholine specificity. The phosphorylcholine-binding ability will facilitate the purification of correctly folded recombinant proteins via a phosphorylcholine affinity column

M E N U

Previous Doc Next Doc Go to Doc# First Hit

Generate Collection

L10: Entry 8 of 43 File: PGPB Mar 7, 2002

DOCUMENT-IDENTIFIER: US 20020028200 A1
TITLE: Anti-idiotypic antibody against FimH adhesion of uropathogenic type I-fimbriated escherichia coli, compositions containing same and method for using same

Detail Description Paragraph:
[0092] Kearney et al, "Monoclonal vs. heterogeneous anti-H-8 antibodies in the analysis of the anti-phosphorylcholine response in BALB/c mice", Eur. J. Immunol. 11:877 (1981)

Previous Doc Next Doc Go to Doc#

Other Reference Publication (18):
Halpern et al., "Human Anti-Phosphorylcholine Antibodies Share Idiotopes and are Self-Binding," J. Clin. Invest., 88:476-482 (1991).

DOCUMENT-IDENTIFIER: US 6632926 B1
TITLE: Antibody variants

Other Reference Publication (25):
Fenney and Thuerauf, "Sequence and fine specificity analysis of primary 511 anti-phosphorylcholine antibodies" Journal of Immunology 143 (12) :4061-4068 (Dec. 15, 1989).

DOCUMENT-IDENTIFIER: US 4203893 A
** See image for Certificate of Correction **
TITLE: Coupling products of cytidine-diphosphocholine and amino-compounds for pharmaceutical use

Brief Summary Text (57):
Reactions of the coupling products cytidine-diphosphocholine-amino compounds with reactive C protein may be inhibited by 5% trisodium nitrate, the product known under the commercial name "Anaklepton" 5.10.sup.-3 M, cytidine-5'-diphosphocholine or phosphorylcholine 10.sup.-3 M, whereas only the two latter compounds inhibit the reaction of the coupling products of the invention with antiphosphorylcholine antibodies, which enables both differential discovery and verification of the specificity of the reaction.

Previous Doc Next Doc Go to Doc#

1: Indian J Pathol Microbiol. 1991 Jul;34(3):176-80. Related Articles, Links

# Antiribitol-teichoic acid antibody (ARTA) in diagnosis of deep seated Staphylococcus aureus infections.

Ayyagari A, Pal N.

Department of Medical Microbiology, Postgraduate Institute of Medical Education & Research, Chandigarh.

Antiribitol-teichoic acid antibody (ARTA) was detected in sera of 30 out of 50 patients (60%) with various acute deep seated Staphylococcus aureus infections and 5 out of 10 chronic osteomyelitis cases, whereas none of the sera from 50 patients with superficial Staphylococcus aureus infections as well from 50 patients without Staphylococcus aureus infections showed antibody response (p less than 0.01). This test is a definite advantage in diagnosis of deep seated staphylococcal infections like endocarditis, lung disease, meningitis and specially in osteomyelitis cases where organisms cannot be isolated and therefore helps in predicting the need for long term antimicrobial therapy.

PMID: 1818853 [PubMed - indexed for MEDLINE]

1: Res Vet Sci. 1976 Nov;21(3):284-91. Related Articles, Links

# Characteristics of Staphylococcus aureus strains isolated from different animal species.

Devriese LA, Oeding P.

Staphylococcus aureus strains originating from humans, cows, poultry, pigs, dogs and pigeons were characterised according to the biotyping scheme of Hajek and Marsalek (1971). All strains obtained from poultry, dogs and pigeons and the majority of bovine, human and porcine strains were classifiable as belonging to different biotypes. Two types were found to be present among poultry strains isolated in Europe and Japan. The porcine strains formed a heterogenic collection. One biotype predominated in the other host species. The characteristic S aureus wall teichoic acid (beta-N-acetylglucosaminyl ribitol teichoic acid) was present in nearly all poultry and pig strains. Strains from dogs and pigeons were found to present several properties which were not in agreement with the species description given for S aureus. They did not produce acetoin from glucose and their capacity to produce acid from mannitol in anaerobic conditions was very weak or absent. They were often negative in the clumping factor (slide coagulase) test and usually did not produce hyaluronidase. The production of acid from glucose in anaerobic conditions was slower and less intensive in these strains than in the S aureus strains from other origins. The results of this study support the concept of subdividing the species S aureus into biotypes or ecotypes.

PMID: 140452 [PubMed - indexed for MEDLINE]

DOCUMENT-IDENTIFIER: WO 9012632 A1
TITLE: PROCESS FOR REMOVING C-REACTIVE PROTEIN AND ANTI-PHOSPHORYLCHOLINE ANTIBODIES FROM BIOLOGICAL FLUIDS

Previous Doc Next Doc Go to Doc#

L7: Entry 13 of 30 File: PGPB Jun 12, 2003

DOCUMENT-IDENTIFIER: US 20030108957 A1
TITLE: Biocidal molecules, macromolecular targets and methods of production and use

Detail Description Paragraph:
[0140] Molecules or compounds that penetrate the peptidoglycan layer of a bacterial cell wall can be constructed from a peptide selected from the pyrrhocoricin-apidaecin-drosocin family and a derivative or analog thereof that binds to the HSP or DnaK present in the lipopolysaccharide layer of Gram-negative bacteria. That peptide is covalently linked to a second compound that has a biological activity within the cell. Methods for making these compounds and for using them in pharmaceutical or veterinary compositions for the treatment of bacterial infections are also part of this invention. Still another aspect of the invention engendered by the discovery that a heat shock protein is the receptor protein of pyrrhocoricin is a molecule that penetrates the peptidoglycan layer of a bacterial cell wall. Gram-negative strains have a cell peptidoglycan wall that is thinner than that of Gram-positive bacteria. However, the cell wall of Gram negative bacteria also contains an outer membrane, composed of a lipid bilayer, some proteins and lipopolysaccharide (LPS), that lies above a layer formed of peptidoglycan with teichoic acid polymers dispersed throughout the layer. The acidic character of the peptidoglycan cell wall naturally binds the highly positively charged antibacterial peptides. As predicted from their positive charge, many antibacterial peptides also bind the negatively charged LPS [Vaara, M. (1992) Microbiol Rev., 56: 395-341]. This seems very beneficial because antibacterial activity of certain peptides must be initiated at the bacterial cell surface if the peptides are too large to diffuse across the outer membrane. Nevertheless, the general destabilization of the outer membrane and the ensuing internalization of some positively charged peptides do not necessarily result in killing the microorganisms without additional intracellular effects.

CLAIMS:

11. The method according to claim 10, wherein said antibody is selected from the group consisting of a polyclonal antibody, a recombinant antibody, a monoclonal antibody, a chimeric antibody, a human antibody, a humanized antibody, an antibody or fragment thereof produced by screening phage displays, and mixtures thereof.

DOCUMENT-IDENTIFIER: US 20040228879 A1
TITLE: Combination vaccine against streptococcus pneumoniae and respiratory syncytial virus (RSV)

Detail Description Paragraph:
[0085] Murine IgG to pneumococcal polysaccharides types 6B, 14, 19F and 23F was measured by ELISA in a method adapted from the CDC protocol. This protocol includes the addition of soluble cell wall polysaccharide (CPS) to the sera to inhibit the measurement of CPS antibodies. CPS is a phosphoryl choline containing teichoic acid common to all pneumococci. It is present under the capsule, and antibodies to it are only weakly protective. Since CPS is linked to the capsular polysaccharide, there is usually 0.5 to 1% CPS contaminating the purified capsular polysaccharide used to coat the ELISA plates. Thus, measurement of the CPS antibodies can confound the interpretation ELISA results with respect to the capsular polysaccharide.

Detail Description Paragraph:
[0136] Murine IgG to pneumococcal polysaccharide types 3, 6B, 7F, 14, 19F and 23F was measured by ELISA in a method adapted from the CDC protocol. This protocol includes the addition of soluble cell wall polysaccharide (CPS) to the sera to inhibit the measurement of CPS antibodies. CPS is a phosphoryl-choline containing teichoic acid common to all pneumococci. It is present under the capsule, and antibodies to it are only weakly protective. Since CPS is linked to the capsular polysaccharide, there is usually 0.5 to 1% CPS contaminating the purified capsular polysaccharide used to coat the ELISA plates. Thus, measurement of the CPS antibodies can confound the interpretation ELISA results with respect to the capsular polysaccharide.

DOCUMENT-IDENTIFIER: US 20030175293 A1
TITLE: Choline binding proteins for anti-pneumococcal vaccines

Summary of Invention Paragraph:
[0014] Previous studies have shown that PspA, as well as one other surface exposed protein, LytA, the autolytic amidase, bind to teichoic acid (TA), an integral part of the cell wall of Streptococcus pneumoniae in a choline-dependent manner. TA contains a unique terminal phosphorylcholine moiety. PspA, a protein having a molecular weight of 84 kDa, and which is highly variable, is released from the cell surface with high choline concentration. Lyt, or autolysin, is a 36 kDa protein, which lyses the pneumococcal cell wall (self lysis), but is not released from the cell by growth in high concentrations of choline, by washing in 10% choline, or by growth in ethanolamine. Reports on choline binding proteins include those by Sanchez-Puelles et al Gene 89:69-75 (1990), Briese and Hakenback Eur. J. Biochem. 146:417-427, Yother and White J. of Bacteriol. 176:2976-2985, Sanchez-Beato et al J. of Bacteriol. 177:1098-1103, and Wren Micro. Review Mol. Microbiol. 5:797-803 (1991), which are hereby incorporated by reference in their entirety.

Summary of Invention Paragraph:
[0015] A variety of covalent and non-covalent mechanisms of attachment have been described for proteins decorating the surfaces of gram positive bacteria. Some streptococci and Clostridium sp. have phosphorylcholine as a unique component of the cell wall. This molecule is the terminal constituent of the teichoic acid (C polysaccharide) and lipoteichoic acid (LTA) attached to the cell wall and plasma membrane of these bacteria. A family of choline binding proteins (CPBs) have also been described which serve a variety of cellular functions. These proteins consist of an N-terminal activity domain and a repeated C-terminal signature choline binding domain that anchors these molecules to the surface of the bacteria. This motif has been identified in the C-terminal regions of a secreted glycoprotein from Clostridium acetobutylicum NCIB 88052 [Sanchez-Beato, et al., J. Bacteriol. 177:1098-1103 (1995)], toxins A and B from Clostridium difficile [Von Eichel-Streiber and Sauerborn, Gene 96:107-13 (1990); Von Eichel-Streiber et al., J. Bacteriol. 174:6707-6710 (1992)], a glucan-binding protein from Streptococcus mutans, several glycosyltransferases from Streptococcus downei and S. mutans, the murein hydrolase (LytA) from pneumococcus and pneumococcal lytic phage [Ronda et al., Eur. J. Biochem. 164:621-4 (1987); Diaz et al., J. Bacteriol. 174:5516-25 (1992); Romero et al., Microb. Lett. 108:87-92 (1993); Yother and White, J. Bacteriol. 176:2976-85 (1994)], and a surface antigen (PspA) also from pneumococcus.

Detail Description Paragraph:
[0106] Teichoic acid (TA), an integral part of the cell wall of Streptococcus pneumoniae contains a unique terminal phosphorylcholine moiety. Choline affinity chromatography or Mono-Q Sepharose, a close relative of choline were used to purify the CBPs. It is important to note that initially these proteins were purified from a capsulated strain of pneumococcus that was genetically altered not to produce PspA, a major CBP. The purification schemes are as follows:

CLAIMS:

2. The streptococcal choline binding protein of claim 1, having one or more of a characteristic selected from the group consisting of: c) inhibiting adherence of the bacteria to host cells; d) being reactive with sera from patients infected or recovering from infection with the bacteria; e) being reactive with rabbit antisera generated against pneumococcal proteins isolated from a choline affinity column by elution in at least about 10% choline; and f) being labeled by fluorescein isothiocyanate (FITC) without requiring streptococcal lysis (i.e., in intact bacteria).

14. A monoclonal antibody to the streptococcal choline binding protein of claim 1.

15. An immortal cell line that produces a monoclonal antibody according to claim 14.

L7: Entry 10 of 30 File: PGPB Sep 18, 2003

PGPUB-DOCUMENT-NUMBER: 20030175293
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030175293 A1

TITLE: Choline binding proteins for anti-pneumococcal vaccines

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY |
|---|---|---|---|
| Masure, H. Robert | Germantown | TN | US |
| Rosenow, Carsten I. | Redwood City | CA | US |
| Tuomanen, Elaine | Germantown | TN | US |
| Wizemann, Theresa M. | Germantown | MD | US |

APPL-NO: 09/829382 [PALM]
DATE FILED: April 9, 2001

RELATED-US-APPL-DATA:
child 09829382 A1 20010409
parent division-of 08847065 19970501 US GRANTED
parent-patent 6245335 US
non-provisional-of-provisional 60016632 19960501 US

INT-CL: [07] A61 K 38/00, A61 K 39/09, C07 K 14/00, A61 K 39/02, C07 K 1/00, C07 K 17/00

US-CL-PUBLISHED: 424/190.1; 530/350, 530/825, 424/244.1, 514/12, 930/200
US-CL-CURRENT: 424/190.1; 424/244.1, 514/12, 530/350, 530/825, 930/200

REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

The invention relates to bacterial choline binding proteins (CBPs) which bind choline. Such proteins are particularly desirable for vaccines against appropriate strains of Gram positive bacteria, particularly streptococcus, and more particularly pneumococcus. Also provided are DNA sequences encoding the bacterial choline binding proteins or fragment thereof, antibodies to the bacterial choline binding proteins, pharmaceutical compositions comprising the bacterial choline binding proteins, antibodies to the bacterial choline binding proteins suitable for use in passive immunization, and small molecule inhibitors of choline binding protein mediated adhesion. Methods for diagnosing the presence of the bacterial choline binding protein, or of the bacteria, are also provided. In a specific embodiment, a streptococcal choline binding protein is an enolase, which demonstrates strong affinity for fibronectin.

[0001] The present application claims priority to Provisional Patent Application Serial No. 60/016,632, filed May 1, 1996, pursuant to 35 USC 119(e), the disclosure of which is incorporated herein by reference in its entirety.

Previous Doc Next Doc Go to Doc#

First Hit Fwd Refs Previous Doc Next Doc Go to Doc#

Generate Collection Print

L12: Entry 8 of 28 File: USPT Jan 20, 2004

DOCUMENT-IDENTIFIER: US 6680192 B1
TITLE: Method for producing polymers having a preselected activity

Drawing Description Text (6):
FIG. 3 Amino acid sequence of the V.sub.H regions of 19 mouse monoclonal antibodies with specificity for phosphorylcholine (SEQ ID NOS:1-19). The designation HP indicates that the protein is the product of a hybridoma. The remainder are myeloma proteins. (From Gearhart et al., Nature, 291:29, 1981.)

Other Reference Publication (111):
Gearhart et al., "IgG antibodies to phosphorylcholine exhibit more diversity than their IgM counterparts, " Nature 291:29-34 (1981).

Other Reference Publication (143):
Gearheart, et al., "IgG antibodies to phosphorylcholine exhibit more diversity than their IgM counterparts" Nature 291: 29-34 (1981).

Previous Doc Next Doc Go to Doc#

Curr Eye Res. 1997 Oct;16(10):1036-43. Related Articles, Links

# Immunopathologic features of Staphylococcus epidermidis-induced endophthalmitis in the rat.

Ravindranath RM, Hasan SA, Mondino BJ.

Doris Stein Eye Research Center, Jules Stein Eye Institute, University of California, Los Angeles, CA, USA.

PURPOSE: To investigate the clinical, histopathologic and immunologic responses to Staphylococcus epidermidis endophthalmitis in a rat model. METHODS: Experimental rats received an intravitreal injection of viable S. epidermidis (7000 organisms), while control rats received sterile saline. The clinical scores, cellular infiltrate in vitreous, and levels of serum and vitreous IgM, IgG and IgA to glycerol teichoic acid (GTA), the major antigenic determinant of S. epidermidis cell wall, were all measured from day 1 to day 30 after injection. RESULTS: The ocular inflammation was largely resolved by day 14. The red reflex was abolished in 50% of rats between days 3 and 10. The bacteria were cleared from the vitreous by day 7. In vitreous, the neutrophils peaked at day 1 and decreased by day 7, and plasma cells were seen between days 1 and 3. Presence of B cells (CD45+/CD3-) was confirmed by flow cytometric analysis of pooled vitreous humor. IgM and IgG but not IgA antibodies to GTA were found in vitreous of injected eyes. The peak of anti-GTA IgM was observed in vitreous of S. epidermidis-infected rats on day 1 and declined by day 7. In contrast to vitreous antibodies, serum anti-GTA IgM antibodies were significantly elevated throughout the course of S. epidermidis endophthalmitis. A weak IgG but no IgA response were observed in serum. Anti-GTA antibodies were also found in low level in normal sera but not in normal vitreous. CONCLUSIONS: The vitreous antibodies may be involved in neutrophil-mediated opsonophagocytosis leading to 'spontaneous sterility' of the bacteria, and may play a role in the immunopathogenesis of staphylococcal endophthalmitis in the rat.

PMID: 9330856 [PubMed - indexed for MEDLINE]

Invest Ophthalmol Vis Sci. 1987 Sep;28(9):1553-8. Related Articles, Links

# Corneal antibody levels to ribitol teichoic acid in rabbits immunized with staphylococcal antigens using various routes.

Mondino BJ, Brawman-Mintzer O, Adamu SA.

Although Staphylococcus aureus is an important cause of infectious diseases of the eye and hypersensitivity lesions of the cornea, little is known about ocular immunity to this pathogen. Using an enzyme-linked immunosorbent assay, we measured antibody titers to ribitol teichoic acid, the major antigenic determinant of S. aureus, in corneas as well as serum and tears after immunizing rabbits using the following routes: intradermal injection of cell wall mixed with complete Freund's adjuvant, subconjunctival injection of cell wall mixed with complete Freund's adjuvant, topical application of cell wall to the eye or topical application of viable S. aureus to the eye. IgG titers to ribitol teichoic acid were found consistently in corneas after intradermal and subconjunctival immunization with cell wall and topical immunization with viable S. aureus. After intradermal immunization with cell wall, IgG titers in cornea were higher than tears but lower than serum, which was presumably the source of the IgG antibodies for the cornea. After subconjunctival immunization with cell wall or topical immunization with viable S. aureus, IgG titers in corneas were higher than tears and generally higher than serum, suggesting that the ocular tissues were a local source of IgG. On the other hand, IgA titers to ribitol teichoic acid were found in tears but not in serum and were found only occasionally in corneas, suggesting that IgG responses to staphylococcal antigens may be more important than IgA responses in the cornea. The results of this study suggest that corneal antibodies to ribitol teichoic acid may be influenced by exposure to staphylococcal antigens not only in the external eye but also at sites remote from the eye.

JOURNAL OF BACTERIOLOGY, Feb. 2001, p. 1133–1139 Vol. 183, No. 4
0021-9193/01/$04.00+0 DOI: 10.1128/JB.183.4.1133–1139.2001


# A Novel Serotype-Specific Gene Cassette (*gltA-gltB*) Is Required for Expression of Teichoic Acid-Associated Surface Antigens in *Listeria monocytogenes* of Serotype 4b

XIANG-HE LEI,[1]† FRANZ FIEDLER,[2] ZHENG LAN,[1] AND SOPHIA KATHARIOU[1]*

*Department of Microbiology, University of Hawaii, Honolulu, Hawaii 96822,[1] and Institute for Genetics and Microbiology, University of Munich, Munich, Germany[2]*

Received 16 August 2000/Accepted 6 November 2000

***Listeria monocytogenes*** **serotype 4b strains account for about 40% of sporadic cases and many epidemics of listeriosis. Mutations in a chromosomal locus resulted in loss of reactivity with all three monoclonal antibodies (MAbs) which were specific to serotype 4b and the closely related serotypes 4d and 4e. Here we show that this locus contains a serotype 4b-4d-4e-specific gene cassette (3,071 bp) which consists of two genes, *gltA* and *gltB*, and is flanked by palindromic sequences (51 and 44 nucleotides). Complete loss of reactivity with the three serotype-specific MAbs resulted from insertional inactivation of either *gltA* or *gltB*. The *gltA* and *gltB* mutants were characterized by loss and severe reduction, respectively, of glucose in the teichoic acid, whereas galactose, the other serotype-specific sugar substituent in the teichoic acid, was not affected. Within *L. monocytogenes*, only strains of serotypes 4b, 4d, and 4e harbored the *gltA-gltB* cassette, whereas coding sequences on either side of the cassette were conserved among all serotypes. Comparative genomic analysis of a serotype 1/2b strain showed that the 3,071-bp *gltA-gltB* cassette was replaced by a much shorter (528-bp) and unrelated region, flanked by inverted repeats similar to their counterparts in serotype 4b. These findings indicate that in the evolution of different serotypes of *L. monocytogenes*, this site in the genome has become occupied by serotype-specific sequences which, in the case of serotype 4b, are essential for expression of serotype-specific surface antigens and presence of glucose substituents in the teichoic acids in the cell wall.**

Numerous serotypes of *Listeria monocytogenes* have been identified using the antigenic scheme of Seeliger and Hoehne (16). However, three serotypes, 1/2a, 1/2b, and 4b, account for more than 95% of clinical isolates (5). Serotype 4b is of special interest, as it is implicated in about 40% of sporadic cases and the majority of epidemics of food-borne listeriosis reported in Europe and North America during the past 20 years (1, 7, 15). This may reflect relatively high virulence of serotype 4b strains for humans, although unique pathogenesis attributes of this serotype have not yet been identified.

The somatic component of the serotypic designation in *Listeria* resides primarily in the anionic polymer, teichoic acid (TA), which consists of polyribitol phosphate and is covalently linked to peptidoglycan (4, 6, 18). Glycosidic substitution(s) of the ribitol phosphate units render the TA variable, structurally and antigenically, among different serotypes. In serogroup 1/2 (e.g., serotypes 1/2a and 1/2b), *N*-acetylglucosamine and rhamnose are present as substituents on the ribitol, whereas in serogroup 4, *N*-acetylglucosamine is integral to the TA chains. A unique glycosidic substitution pattern is present in serotype 4b, where the integral *N*-acetylglucosamine bears both galactose and glucose substituents (4, 18).

In an effort to develop tools useful for the identification of antigenic and genetic attributes unique to serotype 4b bacteria, we have used monoclonal antibodies (MAbs) (c74.22, c74.33, and c74.180) which reacted with strains of serotypes 4b, 4d, and 4e (referred to collectively as serotype 4b-4d-4e) (8) to identify serotype-specific genomic regions. One such region was shown to harbor the serogroup 4-specific gene *gtcA*, which has been recently described (14). Insertional inactivation of *gtcA* resulted in loss of reactivity with one of the MAbs (c74.22), loss of galactose, and marked reductions in the glucose in the TA of the cell (14). A different genomic region was found to be specific to serotypes 4b, 4d, and 4e, and mutants in this region lacked reactivity with all three MAbs (10). Here we report the cloning and characterization of the genes composing this region and provide genetic evidence for their involvement in serotype-specific surface antigen expression and TA glycosylation in *L. monocytogenes* serotype 4b.

## MATERIALS AND METHODS

**Bacterial strains and media.** *Listeria* and *Escherichia coli* strains were grown and preserved as described before (14). Antibiotics used for *Listeria* and for *E. coli* were as described before (14). Generation of transposon mutants of the serotype 4b strain 4b1 and screening of the mutants with the MAbs have been described elsewhere (10).

**Biochemical analysis of cell wall composition.** Cell wall composition was determined as described by Fiedler et al. (4). TA from *Listeria* was prepared and analyzed as previously described (4, 6).

**Molecular procedures.** Procedures for extraction of plasmid DNA from *E. coli* and genomic DNA from *Listeria* and for nonradioactive labeling and detection of DNA were previously described (10). Fragment XL7-1, which flanks the single transposon insertion in mutant XL7, has been described elsewhere (10). This fragment was sequenced, and inverse PCR (13) was employed to obtain genomic fragments on either side, using as template genomic DNA of the wild-type strain 4b1 digested with *Eco*RI or *Sau*3A, purified from low-melting-point agarose with phenol-chloroform extractions (2), and self-ligated. Amplified fragments were cloned in pCR2.1 (Invitrogen) and sequenced. Sequence information was used to design new primers at the end of the known sequence for additional inverse

* Corresponding author. Present address: Department of Food Science, Food Pathogens Laboratory, North Carolina State University, Raleigh, NC 27695. Phone: (919) 513-2075. Fax: (919) 515-7124. E-mail: skathar@unity.ncsu.edu.

† Present address: Biolog, Hayward, CA 94545.

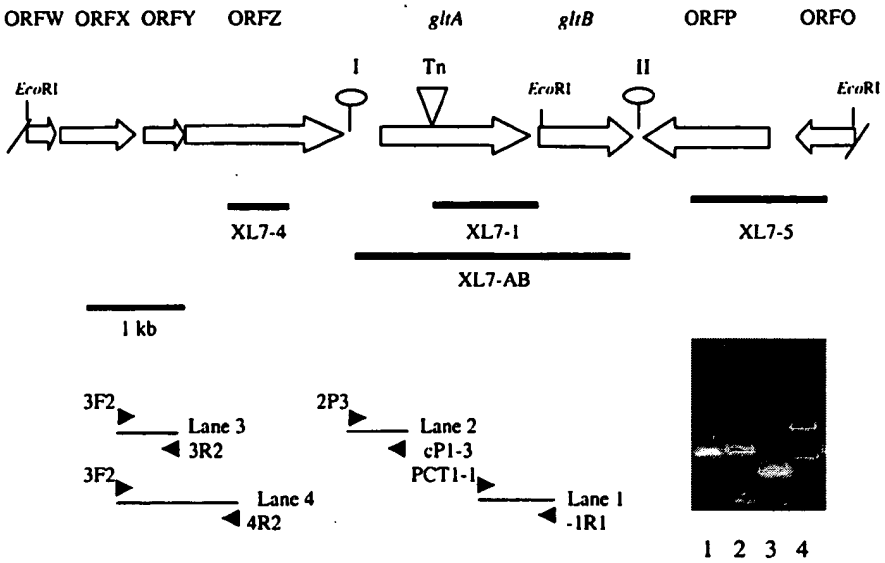



FIG. 1. Genomic organization of the region harboring the transposon insertion in mutant XL7. Open arrows indicate ORFs and predicted direction of transcription. Slashes at the borders indicate that ORFW and ORFO are partial; lollipops represent putative stem-loop structures. The location of the Tn*916*ΔE insertion in *gltA* (Tn) is indicated by a triangle. Thick lines represent DNA fragments used as probes in Southern blots, and arrowheads at the bottom indicate primers used in RT-PCR. RT-PCR was done as described in Materials and Methods with -1R1 as the primer for cDNA synthesis. The gel shows products of PCRs with cDNA as the template and the primer pairs -1R1–PCT1-1 (lane 1), cP1-3–2P3 (lane 2), 3R2-3F2 (lane 3), and 4R2-3F2 (lane 4). Negative controls (using RNA instead of cDNA as the template and the same pairs of primers) were devoid of any product (data not shown).

PCRs. Transposon-flanking fragments from other mutants were amplified using the Tn*916* terminal primer OTL (5′-CGG AAT TCC GTG AAG TAT CTT CCT ACA G-3′) with a 5′-end *Eco*RI site (underlined) and primer cP1 (5′-CAC AGA AGC GAT ACG ATG A-3′).

**Probe construction.** Probe locations are shown in Fig. 1. Probe XL7-1 (1.1 kb), which flanks the transposon insertion in mutant XL7, has been described elsewhere (10). Probe XL7-4 (0.6 kb) is internal to open reading frame Z (ORFZ) and consists of a 0.6-kb *Sau*3A fragment cloned into pUC19. Probe XL7-5 (1.6 kb), which includes ORFP and part of ORFO, was obtained as a PCR fragment with primers −1F5 (5′ CCG ACT GTA TCT TCT TTT CC 3′) and −1R9 (5′ TTT GCT ACT CAA CGG AGC CAC 3′) and 4b1 DNA as the template. The XL7-AB probe (2.9 kb), which includes both *gltA* and *gltB*, was obtained as a PCR fragment using primers 2P3 (5′-GTA ACG TCT CAT ATA GGG AG-3′) and −1R5 (5′-GTA GAA CAA TTG TAG TAC CG-3′). DNA fragments were isolated from low-melting-point agarose gels, purified by phenol-chloroform extractions (2), and labeled with a Genius kit.

**RT-PCR.** Procedures for RNA extraction from *Listeria*, construction of cDNA, and reverse transcription PCR (RT-PCR) were as described elsewhere (14).

**Construction of integration mutant in *gltB*.** To construct an integration mutant in *gltB*, an internal fragment of the gene was cloned in the temperature-sensitive shuttle vector pKSV7 (17), and integrants were selected by growth at the restrictive temperature (43°C) in medium containing chloramphenicol (CM medium) as follows. The internal fragment was amplified with primers −1F2 (5′-TTG GTA ACT CAC TAG TAC GT) and −1R4 (5′-ACA AGC ACA AAC AAA GAC GC), cloned in pCR2.1, recovered by *Eco*RI digestion, and subcloned into *Eco*RI-digested and dephosphorylated pKSV7. The resulting recombinant was electroporated into electrocompetent cells of the parental strain 4b1 as previously described (14), and transformants were isolated on CM medium after 48 h at 30°C. Integrants were isolated following four consecutive passages in CM medium at the restrictive temperature (43°C) and confirmed by Southern blotting. For colony immunoblots, the cultures were grown at room temperature.

**Construction of pKA and pKAB.** *Listeria* DNA fragments harboring *gltA* and *gltA-gltB* were amplified from DNA of the parental strain 4b1 by PCR using High Fidelity enzyme (Roche). Fragment A (containing *gltA*) was obtained by PCR using primers 2P3 and −1R1 (5′-CAA GGC AAG AGT ACA GCT AC-3′). Fragment AB (containing *gltA-gltB*) was amplified using primers 2P3 and −1R5 (described above for construction of the *gltA-gltB* probe XL7-AB), which had a *Hin*dIII site and a *Bam*HI site, respectively, at the 5′ end. The PCR fragments were excised from low-melting-point agarose gels, purified with phenol-chloroform, and cloned into pCR2.1. Fragment A was isolated following digestion of the recombinant plasmid with *Eco*RI and was subcloned into pKSV7 which had been digested by *Eco*RI and dephosphorylated. Fragment AB was obtained following digestion of the plasmid with *Bam*HI and *Hin*dIII and directionally cloned into pKSV7 digested with the same enzymes. The resulting plasmids, consisting of pKSV7 with inserts of *gltA* and *gltA-gltB*, were named pKA and pKAB, respectively. Upon electroporation, 100 μl of the cells was plated on CM medium, and the plates were incubated at 30°C for 3 to 4 days.

**Cloning of serotype 1/2b sequences.** Primers 2P2 (5′- GAC CAT ATC GTC GTG CTA CA-3′) and −1R65 (5′-CGA GCA TAC AAG TGC TCG TT-3′) were used to amplify a 1.1-kb DNA fragment with DNA of strain F4242 (serotype 1/2b) as the template. The 1.1-kb PCR product was directly cloned into pCR2.1 and sequenced on both strands.

**DNA sequencing and sequence analysis.** Nested deletions were generated using the Erase-a-Base system (Promega) as suggested by the vendor. DNA was sequenced and analyzed as previously described (14).

**Nucleotide sequence accession number.** The nucleotide sequence data for *L. monocytogenes* serotypes 4b and 1/2b have been deposited in GenBank under accession numbers AF033015 and AF033016, respectively.

## RESULTS

**Mutants negative for serotype-specific MAbs.** The single-insertion Tn*916*ΔE mutant XL7 lacked reactivity with all three serotype-specific MAbs (C74.22, C74.33, and C74.180) but had no readily detectable phenotypic differences from its wild-type counterparts in terms of growth at 20 and 35°C, motility, sensitivity to serotype-specific phage 2671 or *Listeria*-specific phage A511, hemolytic activity, and colony or cellular morphology. Furthermore, four additional independent transposon mutants (33N1, 33N2, 33N3, and 8A3) phenotypically identical to XL7 were found to harbor transposon insertions in the same *Eco*RI and *Hin*dIII genomic fragment as XL7 (10), suggesting that the MAb-negative phenotype of XL7 was associated with the Tn*916*ΔE insertion. DNA sequence analysis of XL7-1 and of the additional fragments derived by inverse PCR showed that the transposon was inserted in an ORF

termed *gltA* (for glucose in teichoic acid). The transposon insertion sites in mutants 33N1, 33N2, 33N3, and 8A3 were within a 10-nucleotide (nt) region in *gltA*, which also harbored the insertion in XL7. The target sequence for the transposon insertions conformed to the consensus target sequence (T[T/A ]TTTTNNNNNNAAAAA[A/T]A) for Tn*916* (11).

**Genomic organization and ORF analysis of the *gltA-gltB* region.** Sequence analysis revealed six complete ORFs (ORFX, ORFY, ORFZ, *gltA*, *gltB*, and ORFP) and two partial ORFs (ORFW and ORFO) in this region (Fig. 1). ORFW (partial), ORFX, ORFY, ORFZ, *gltA*, and *gltB* were transcribed in the same direction and convergently to ORFP and ORFO (partial). Two palindromic sequences with the potential to form pronounced stem-loop structures flanked the *gltA-gltB* region. The palindrome for putative stem-loop I (51 nt; calculated free energy of formation, −46 kcal/mol) was in the region between ORFZ and *gltA*, 55 nt downstream of ORFZ and 279 nt upstream of *gltA*, whereas that for putative stem-loop II (44 nt; calculated free energy of formation also −46 kcal/mol) was 8 and 27 nt downstream of *gltB* and ORFP, respectively (Fig. 1). The organization of the region suggests that stem-loops I and II may serve as transcription terminators for ORFZ and ORFP, respectively.

The G+C contents of *gltA* and *gltB* were 34 and 34.8%, respectively, lower than is typical for *L. monocytogenes* (38%). In contrast, the other ORFs in this region had G+C contents noticeably higher than those of *gltA* and *gltB*: ORFW, ORFX, ORFY, and ORFZ had G+C contents of 38.8, 39.7, 41.5, and 39.8%, respectively, whereas the values for ORFP and ORFO were 39.6 and 40.6%, respectively.

***gltA-gltB* region.** The transposon-harboring ORF (*gltA*) (1,647 bp) was 386 nt downstream of ORFZ. We were unable to identify sequences upstream of *gltA* with detectable similarity to the canonical Shine-Dalgarno ribosome recognition sequences. A putative −10 promoter element (TATTAT) was identified 92 nt upstream of the putative start codon of *gltA*. The coding sequence of *gltA* appears to be novel, as screens of the nucleotide and protein databases failed to identify sequences with significant homology to either the gene or the deduced gene product. The latter (548 amino acids, calculated $M_r$ of 62,755, pI 9.0) may be membrane associated in *L. monocytogenes*, as hydrophobicity analysis of the deduced polypeptide revealed 11 putative transmembrane segments (data not shown).

Immediately downstream of *gltA* was *gltB* (948 nt). The *gltA-gltB* intergenic space was only 10 nt, and the putative Shine-Dalgarno site preceding *gltB* (AGGAGAGA) included the last nucleotide of the *gltA* ochre codon, suggesting that the two ORFs may be translationally coupled. *gltB* had 57% identity over its entire length with *rfbj* (ORF10X5) and ORF10X9, which are adjacent to each other on the genome of *Shigella flexneri* (accession no. X71970). In *S. flexneri* this region has been shown to be involved in polymerization of lipopolysaccharide (12), although the exact functions of these two ORFs are unknown.

The deduced *gltB* gene product (315 amino acids, calculated $M_r$ of 36,223, pI 6.04) contained two putative transmembrane domains (underlined in Fig. 2). Protein database searches showed significant similarity between the putative GltB and the deduced products of the *S. flexneri rfbj* and ORF10X9 (56 and 42% identity, respectively). The putative GltB also had 48% identity over its entire length with RfbJ of *Synechocystis* sp. strain PCC6803 (accession no. S77381) and lower (25 to 35%) identity with numerous glycosyltransferases and dolichol phosphate mannosyltransferases from bacteria and archaea. Figure 2 shows alignment of the deduced *gltB* gene product sequence with selected sequences.

**Coding sequences upstream of *gltA-gltB* (ORFW to ORFZ).** BLAST and motif search analysis of the deduced amino acid sequences of ORFW (partial), ORFX, ORFY, and ORFZ suggested that all had characteristics of ABC (ATP-binding cassette) transporters (3). A putative ATP/GTP-binding site motif A (P loop) was identified in the deduced sequences of ORFX (residues 217 to 225) and ORFZ (residues 368 to 375). The ORFW-ORFX and ORFX-ORFY intergenic spaces were 2 and 21 nt, respectively, whereas the stop codon of ORFY overlapped by one nucleotide with the putative start codon of ORFZ, suggesting that ORFY and ORFZ are translationally coupled.

**Coding sequences downstream of *gltA-gltB* (ORFP and ORFO).** FASTA and BLAST analysis of ORFP, located downstream of *gltB* and transcribed convergently, suggested that the deduced product may be a penicillin-binding protein (PBP), having 34 to 55% identity over the entire amino acid sequence with PBPs from numerous other bacteria. Highest similarity (55% identity) was observed with the D-alanyl-D-alanine carboxypeptidase, PBP5, of *Bacillus subtilis* (accession no. P08750). ORFP was preceded by ORFO (partial), transcribed in the same orientation as ORFP and separated from it by 214 nt. The deduced ORFO product had 49 and 46% identity over its entire available length (189 amino acids) with the 7-β-(4-carboxybutanamido)cephalosporanic acid acylase (glutaryl 7-amino cephalosporanic acid [7-ACA] acylase precursor) of *Bacillus laterosporus* and with the cocaine esterase of *Rhodococcus* sp. strain MB1, respectively. These similarities are difficult to evaluate at this time, as such enzymatic activities have not been detected before in *L. monocytogenes*.

**Transcriptional studies.** The quantitative levels of *gltA-gltB* transcripts were too low for reliable detection and size determination by Northern blotting (data not shown), and RT-PCR was used for transcriptional studies. When primer −1R1 (located in *gltB*) was used for reverse transcription, a PCR product of the expected size was obtained using primers −1R1 and PCT1-1 (spanning *gltB* and *gltA*) (Fig. 1, lane 1), suggesting that *gltA* and *gltB* were cotranscribed. Furthermore, the transcript contained the 386-nt region between *gltA* and ORFZ, which includes the palindromic sequence, as suggested by a product of the expected size with primers cP1-3 and 2P3 (located in the region between ORFZ and *gltA*) (Fig. 1, lane 2). Surprisingly, cDNA produced by primer −1R1 could be amplified by primer 3R2 (located in the 3′ region of ORFX) and either 3R2 or 4R2 (Fig. 1, lanes 3 and 4), suggesting that ORFY and ORFZ were included in the transcript as well. These and additional RT-PCR data (not shown) suggest the presence of transcripts harboring not only *gltA-gltB* but also extending through the relatively long (386-nt) region between *gltA* and ORFZ, to at least 2,287 nt upstream of *gltA*. *gltB* appears to be the last ORF in this transcriptional unit.

**Insertional inactivation of either *gltA* or *gltB* results in absence of glucose in the TA, whereas galactose is not affected.**

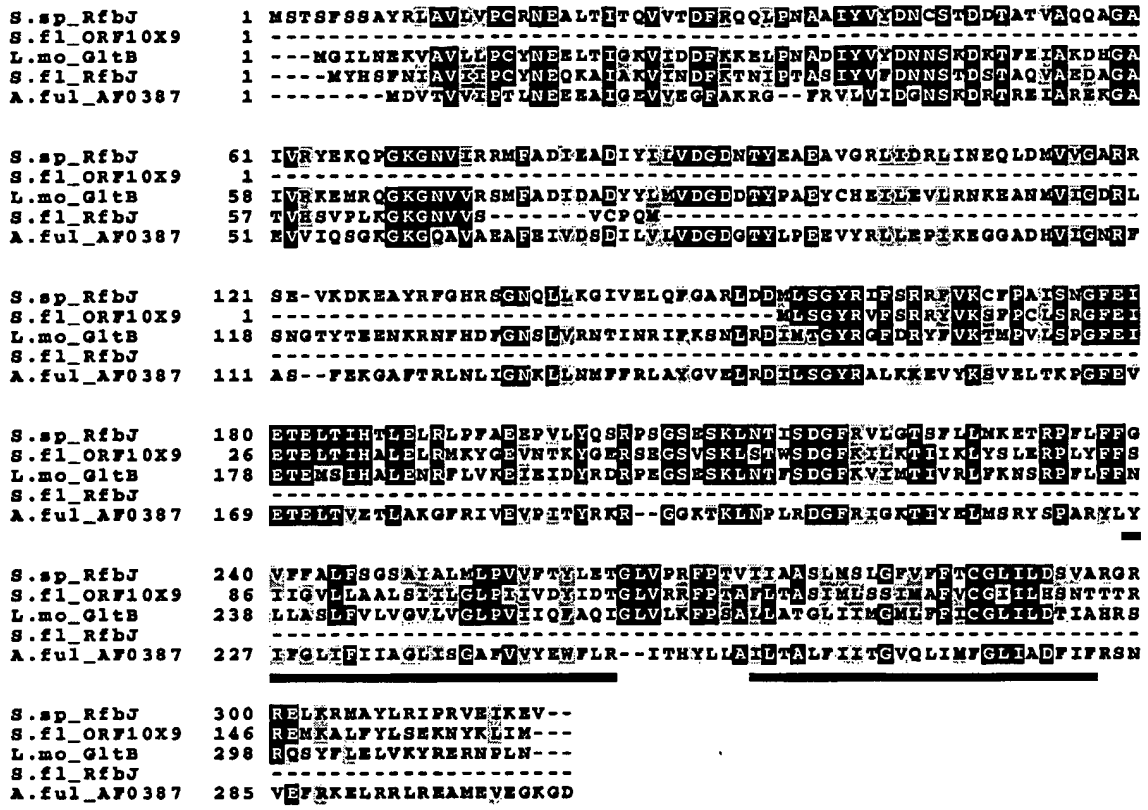



FIG. 2. Multiple sequence alignment (CLUSTAL) of the deduced sequences of, from top to bottom, RfbJ of *Synechocystis* sp. strain PCC6803 (accession no. S77381), ORF10X9 of *S. flexneri* (accession no. P37787), GltB of *L. monocytogenes* (accession no. AF033015), RfbJ of *S. flexneri* (accession no. P37786), and a putative glycosyltransferase of *Archaeoglobus fulgidus* (accession no. AE001078). The underlined segments represent predicted transmembrane regions.

Transposon mutants in *gltB* were not identified, and an integration mutant (4b1-INTB) was constructed, using the temperature-sensitive plasmid pKSV7. Similarly to XL7, the mutant had normal growth and other phenotypic characteristics but lacked reactivity with all three MAbs (data not shown). Biochemical analysis of TA from XL7 and 4b1-INTB showed that both mutants were severely deficient in glucose. In contrast to the wild-type parental strain 4b1, which had both galactose and glucose as substituents on the *N*-acetylglucosamine of the TA, as is typical of serotype 4b (4), glucose was undetectable in the TA of XL7 and present in only trace amounts in the TA of 4b1-INTB (Fig. 3). Interestingly, the other serotype-specific substituent, galactose, was present in normal amounts in the TA of the mutants, as were the integral components of TA (ribitol phosphate and *N*-acetylglucosamine) (Fig. 3). The loss of glucose in the TA of XL7 was also seen with the independently obtained *gltA* mutants 33N1, 33N2, 33N3, and 8A3 (data not shown).

**The MAb-negative phenotype of XL7 can be partially complemented by *gltA* alone or in combination with *gltB*.** The recombinant plasmids pKA and pKAB, harboring *gltA* alone and together with *gltB*, respectively, were electroporated into mutant XL7. Both plasmids included 219 nt upstream of the start codon of *gltA*, since a promoter may be contained within this region. The resulting strains were grown in the presence of chloramphenicol at 30°C, a temperature which permits both replication of the temperature-sensitive plasmid (17) and optimal expression of the serotype-specific surface antigens (8). Reactivity of the mutant with c74.22, c74.33, and c74.180 was restored partially and to the same levels by both plasmids, whereas XL7 harboring the shuttle vector pKSV7 alone remained negative with the MAbs (data not shown). Although pKA and pKAB partially restored reactivity with the MAbs, glucose in the TA of the mutant was not restored to detectable levels (data not shown).

***gltB* is needed for heterologous expression of the serotype-specific surface antigens in strains of serotypes 4a and 4c.** Strains of serotypes 4a and 4c lacked reactivity with the *gltA*-derived probe XL7-1 (10). When transformed with pKAB, strains ATCC 19114 (serotype 4a) and ATCC 19116 (serotype 4c) were rendered reactive with at least two of the MAbs, c74.22 and c74.33 (data not shown). When transformed by pKA these strains remained MAb negative, suggesting that *gltB* was required for expression of c74.22- and c74.33-specific surface antigens in these heterologous hosts.

**Within *L. monocytogenes*, only strains of serotype 4b-4d-4e harbored sequences with homology to *gltA* and *gltB*, whereas ORFP and ORFZ were conserved among different serotypes.** Hybridizations using probe XL7-AB, which contains both *gltA* and *gltB*, showed that the genes were unique to *L. monocytogenes* serotype 4b-4d-4e and could not be detected in DNA from strains of other serotypes. The genes have also been

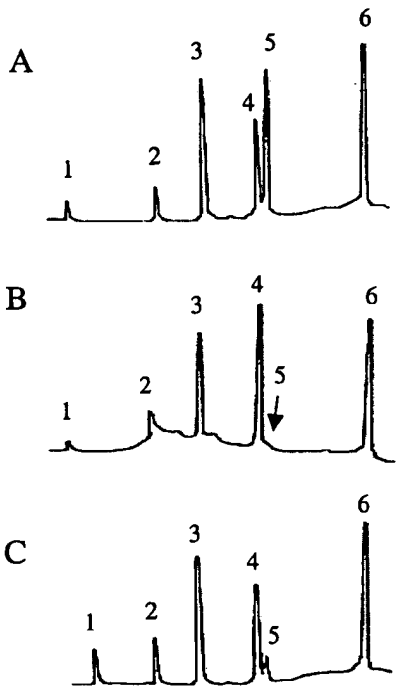



FIG. 3. TA composition of wild-type strain 4b1 (A), *gltA* mutant XL7 (B), and *gltB* mutant 4b1-INTB (C). TA preparation and analysis were done as described previously (4, 6). Peaks: 1, glycerol; 2, anhydroribitol; 3, ribitol; 4, galactose; 5, glucose; 6, glucosamine. The arrow indicates the position of the missing glucose peak in panel B.

detected in a unique lineage (lineage I) of *L. innocua* (9). *Eco*RI restriction fragment length polymorphisms using XL7-AB as the probe could differentiate between *L. monocytogenes* serotype 4b-4d-4e and *L. innocua* lineage I (Table 1). Southern blot and PCR data suggest that the *gltA-gltB* cassette was flanked by ORFZ and ORFP in *L. innocua* lineage I, as in *L. monocytogenes* serotype 4b (data not shown). No hybridization was observed with DNA from other *L. innocua* strains or other *Listeria* species (Table 1).

Southern blots using probes derived from ORFP-ORFO and ORFZ hybridized with all screened serotypes of *L. monocytogenes* suggesting that, in contrast to *gltA* and *gltB*, these sequences were conserved among different serotypes (Fig. 4 and 5). *Eco*RI restriction fragment length polymorphisms could be detected with probes derived from ORFZ and ORFP (Table 1). Sequences homologous to the ORFP- and ORFZ-derived probes were detected in other *Listeria* species as well, except for *L. grayi* and *L. welshimeri* (Table 1).

**Serotype 1/2b *L. monocytogenes* harbors a novel locus genomically equivalent to the *gltA-gltB* cassette of serotype 4b-4d-4e.** The genomic equivalent of the region flanked by the conserved ORFZ and ORFP was amplified from strain F4242 (serotype 1/2b) as described in Materials and Methods. In serotype 1/2b, ORFP and ORFZ flanked a region of 528 bp, in contrast to 3,071 bp in serotype 4b (Fig. 6). Interestingly, the region in serotype 1/2b was flanked by palindromic sequences with significant sequence identity (72 and 84%) to their counterparts in serotype 4b (Fig. 7). The remainder of the 528-bp region, however, showed no detectable homology with the serotype 4b sequences. The 1/2b sequence contained only a small potential coding sequence (ORFC, 75 amino acids), which was preceded by a putative Shine-Dalgarno sequence 7 nt upstream of the putative start codon. The palindromic sequence between ORFC and ORFP was followed by a string of eight T's, suggesting that it may function as a rho-independent terminator. The G+C content of ORFC was unusually low (26%), and no homologous sequences were identified in searches of the DNA and protein databases.

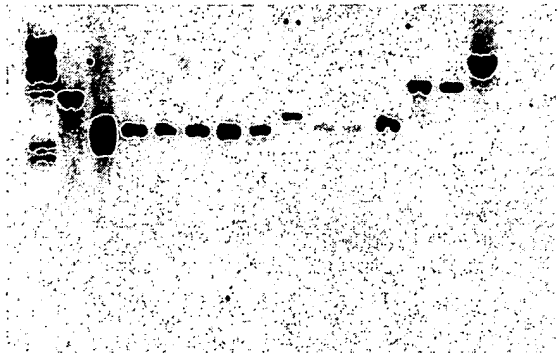
The sequenced 3′ portions of ORFP and ORFZ from serotype 1/2b were 97 and 98%, respectively, identical to their counterparts in serotype 4b. The corresponding C-terminal sequences of ORFP (144 residues) and ORFZ (31 residues) had 98 and 100%, respectively, identity, to their serotype 4b counterparts. Furthermore, Southern blots using probes derived from the regions outside the putative stem-loop structures (ORFP, ORFZ, and sequences distal to them) showed that the corresponding sequences and genomic organization were conserved between serotypes 4b and 1/2b (data not shown). The combined nucleotide sequence and Southern blot data suggest that the genomic organization of this region in serotypes 1/2b and 4b is as shown in Fig. 6.

TABLE 1. Southern blot hybridization data using probes from the *gltA-gltB* genomic region and *Eco*RI-digested DNAs from different strains of *Listeria*

| Strain (serotype) | Hybridization with indicated DNA probe[a] | | | |
|---|---|---|---|---|
| | XL7-4 (ORFZ) | XL7-1 (*gltA*) | XL7-AB (*gltA-gltB*) | XL7-5 (ORFP-PRFO) |
| *L. monocytogenes* | | | | |
| 4b1 (4b) | 5.0 | 5.0 | 5.0, 3.0 | 3.0 |
| F2381 (4b) | 4.5 | 4.5 | 4.5, 3.0 | 3.0 |
| G2228 (1/2a) | 2.6 | 0 | 0 | 6.0 |
| F4242 (1/2b) | 2.6 | 0 | 0 | 2.6 |
| F4245 (1/2b) | 2.6 | 0 | 0 | 5.0 |
| LM103 (1/2c) | 2.6 | 0 | 0 | 2.6 |
| ATCC 19113 (3a) | 2.6 | 0 | 0 | 2.6 |
| G3331 (3b) | 2.6 | 0 | 0 | 2.6 |
| ATCC 2540 (3b) | 3.0 | 0 | 0 | 2.6 |
| G4315 (3c) | 2.6 | 0 | 0 | 2.6 |
| SLCC 2479 (3c) | 2.6 | 0 | 0 | 2.6 |
| ATCC 19114 (4a) | 2.8 | 0 | 0 | 6.5 |
| ATCC 19116 (4c) | 6.5 | 0 | 0 | 6.5 |
| ATCC 19117 (4d) | 5.0 | 5.0 | 5.0, 3.0 | 3.0 |
| ATCC 19118 (4e) | 4.5 | 4.5 | 4.5, 3.2 | 3.2 |
| G2940 (4ab) | 7.5 | 0 | 0 | 1.5 (weak) |
| SLCC 2480 (7) | 2.6 | 0 | 0 | 2.6 |
| *L. innocua* | | | | |
| 120A1 | 4.2 | 0 | 0 | 1.5 |
| F8596[b] | 3.0 | 2.2 | 2.2, 1.7 | 1.5 |
| G6882 | 4.2, 3.0 | 0 | 0 | 5.0, 1.5 |
| G803 | 4.2, 3.0 | 0 | 0 | 5.0, 1.5 |
| *L. grayi* | 0 | 0 | 0 | 0 |
| *L. ivanovii* | 4.0 | 0 | 0 | 1.5 |
| *L. seeligeri* | 7.5 (weak) | 0 | 0 | 7.5 (weak) |
| *L. welshimeri* | 0 | 0 | 0 | 0 |

[a] DNA probes are as indicated in Materials and Methods and in Fig. 2 (thick lines). Values are the sizes of hybridizing fragments (in kilobases).

[b] Member of special lineage of *L. innocua* with serotype 4b-like TA composition (9).



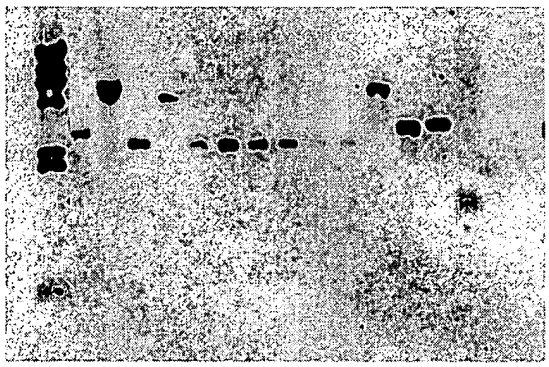
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

FIG. 4. Southern blot of *Eco*RI-digested genomic DNAs from *L. monocytogenes* of different serotypes, using the ORFZ-derived fragment XL7-4 (Fig. 1) as the probe. Lane 1, λ *Hin*dIII-digested molecular size markers (fragment sizes [from the top to bottom], 23, 9.4, 6.6, 4.4, 2.3, 2.0, and 0.56 kb); lanes 2 to 15, *L. monocytogenes* F2381 (4b), G2228 (1/2a), F4242 (1/2b), F4254 (1/2b), LM103 (1/2c), ATCC 19113 (3a), G3331 (3b), SLCC 2540 (3b), G4315 (3c), SLCC 2479 (3c), ATCC 19114 (4a), ATCC 19117(4d), ATCC 19118 (4e), and G2940 (4ab), respectively; lanes 16 and 17, *B. subtilis* 168 and *B. subtilis* W23, respectively. Lanes 10 and 11 contained relatively low amounts of DNA.

## DISCUSSION

The ca. 3-kb gene cassette described here represents a novel serotype-specific locus present in serotype 4b *L. monocytogenes* and the genetically closely related (albeit relatively rare) serotypes 4d and 4e but in no other serotypes of the species. In addition, a unique *L. innocua* lineage that reacts with the serotype 4b-4d-4e-specific MAbs (9) also harbors the cassette, in the same genomic location as serotype 4b *L. monocytogenes*. The distribution of the cassette in *Listeria* parallels precisely the pattern of reactivity of the serotype-specific MAbs (8).

The genes on either side of the cassette were found to be conserved among different serotypes of *L. monocytogenes* as well as other *Listeria* species (*L. innocua, L. ivanovii*, and *L. seeligeri*). On one side, at least one of these genes (ORFP)



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

FIG. 5. Southern blot of *Eco*RI-digested genomic DNAs from *L. monocytogenes* of different serotypes, using the ORFP-ORFO-derived fragment XL7-5 (Fig. 1) as the probe. Lanes are identical to those in Fig. 4. The membrane used for the Southern blot in Fig. 4 was stripped of its probe and reprobed with XL7-5.

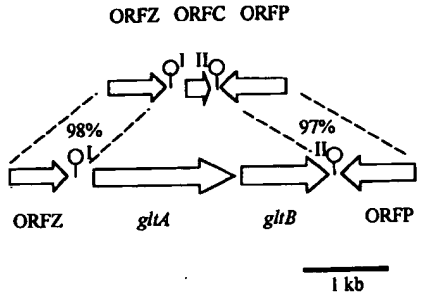



FIG. 6. Comparison between the *gltA-gltB* region of serotype 4b (bottom) and the genomically equivalent region in serotype 1/2b (top). Putative stem-loops I and II (lollipops) represent the boundaries of unique cassettes of different size (3,071 bp in serotype 4b and 528 bp in serotype 1/2b). The percentages indicate nucleotide sequence identities between the corresponding conserved ORFs on either side of the cassettes.

may be involved in cell wall biosynthesis, the deduced product being a putative PBP. On the other side, we identified four genes with homology to ABC transporters. It remains to be determined whether the products of these genes mediate transport of cell wall or TA precursors.

The serotype-specific distribution of the cassette and its unusually low (for *Listeria*) G+C content suggest the possibility that it may have been introduced to the *L. monocytogenes* serotype 4b-4d-4e lineage by horizontal transfer from some unidentified source. From there it could have been transferred to lineage I of *L. innocua*, as has been speculated for the *gtcA* locus recently identified in this lineage (9). The origin of the serotype-specific sequences may be elucidated by future identification of homologous sequences in other bacteria or bacteriophage. It is tempting to speculate that the inverted repeats flanking the serotype-specific sequences may represent remnants of a genetic system (e.g., a transposon or phage) that may have mediated this transfer. These palindromic sequences may have assumed novel functions in their current locations in serotype 4b *L. monocytogenes*, possibly related to transcriptional termination, message stability, or other regulatory mechanisms. Interestingly, these inverted repeats were similar to their counterparts in serotype 1/2b. In the latter, however, the genomic location of the ca. 3-kb serotype 4b-4d-4e-specific cassette was occupied by a much shorter (528-bp) region, which harbors a novel, unrelated ORF. Involvement of the 1/2b region (ORFC) in expression of surface antigen(s) in serotype 1/2b remains to be determined.

```
                    I
1/2b    122 AAAGTGAGTTCTTACGAGATTTTAGTAAGGACTCACTTT 160
            |||| |||| ||||     ||  ||||||| ||| ||||
4b     3350 AAAGCGAGTCCTTATCTTTTTCAAGTAAGGGCTCGCTTT 3388

                    II
1/2b    584 AAGAAGGTCGATTTCCTAATT.AGGAAATCGACCTTCTT 621
            || |  ||||||||||||||| ||||||||||  | ||
4b     6290 AAAAGAGTCGATTTCCTAATTCAGGAAATCGACTCTTTT 6328
```

FIG. 7. Comparison (BESTFIT) of the palindromic sequences corresponding to the putative stem-loops I and II in *L. monocytogenes* of serotypes 4b and 1/2b. Locations of putative stem-loops are as indicated in Fig. 6.

The integrated genetic, immunological, and biochemical results suggest that in *L. monocytogenes* serotype 4b, the *gltA-gltB* cassette is involved in expression of the surface antigens recognized by MAbs c74.22, c74.33, and c74.180 and in the addition of glucose substituents on the TA, but the precise biochemical functions of the two genes remain to be elucidated. The genes can be cotranscribed, and at this time we cannot exclude the possibility that the transposon insertion in *gltA* may have polar effects on *gltB*. The fact that the observed phenotypic complementation of XL7, albeit partial, was conferred equally by pKA and pKAB suggests that *gltB* was expressed to some extent in this mutant. Construction of alternative mutants in *gltA* (such as an in-frame deletion) and/or alternative complementation strategies will be needed to more precisely address the function(s) of *gltA*. Sequence analysis could not facilitate functional predictions in the case of *gltA*, as both the gene and the deduced gene product appeared to lack homologs in the databases. The deduced *gltB* product, however, had significant similarity with numerous glycosylases and dolichol phosphate mannosyltransferases, and a glycosylase function would be in agreement with the observed deficiency of glucose in the TA of the *gltB* mutant.

Complementation of MAb reactivity of XL7 by *gltA* or *gltA-gltB* was partial, for reasons that are not clear but may involve absence of possibly required *cis* elements or suboptimal copy number of the genes in the vector that was used. The low level of complementation may account for the lack of detectable restoration of glucose in the TAs. Such difficulties with complementation were not experienced with the previously studied gene *gtcA*, where both MAb reactivity and TA glycosylation were restored by the wild-type gene in *trans* (14). The mechanisms controlling regulation of expression of *gltA* and *gltB* are not understood but may be complicated, as suggested by the presence of the long and apparently transcribed region between ORFZ and *gltA*.

Glycosylated TA components have been shown to be important antigenic determinants in *L. monocytogenes* (6, 19), although their role in infection has not been elucidated. It is worthy of note that even though *gltA* or *gltB* mutants grew normally in the laboratory, our surveys of numerous serotype 4b field isolates (both food and clinical) failed to identify strains which had the XL7 or 4b1-INTB phenotype or which lacked *gltA-gltB* sequences. One may speculate that because of its surface exposure, abundance, and immunogenicity, properly decorated TA may be important in interactions between the bacteria and their host cells. Glycosylated TA may also affect physiological attributes of the bacteria in foods or in the environment, in response to environmental stresses, association with surfaces and with other organisms in biofilms, etc. Continuing studies in our laboratory aim toward further elucidation of the serotype-specific gene cassettes described in this report in terms of their evolution and potential roles in adaptive physiology and pathogenesis of the listerial lineages which harbor them.

## ACKNOWLEDGMENTS

This research was partially supported by U.S. Department of Agriculture Competitive Research Initiative AAFS grant 99-35201-8183 and by ILSI North America.

We are grateful to Huyen Le Tran for assistance with graphics and to Vladimir Lazarevic for exchange of information related to teichoic acid biosynthesis genes. We thank Nattawan Promadej, Wei Zheng, Edward Lanwermeyer, and all other members of our laboratories for valuable feedback and support throughout the course of this work.

## REFERENCES

1. **Anonymous.** 1999. Update: multistate outbreak of listeriosis—United States, 1998–1999. Morbid. Mortal. Wkly. Rep. **47**:1117–1118.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. D. Seidman, J. A. Smith, and K. Struhl (ed.).** 1987. Current protocols in molecular biology. Greene Publishing Associates and John Wiley & Sons, Inc., New York, N.Y.
3. **Fath, M. J., and R. Kolter.** 1993. ABC transporters: bacterial exporters. Microbiol. Rev. **57**:995–1017.
4. **Fiedler, F., J. Seger, A. Schrettenbrunner, and H. P. R. Seeliger.** 1983. The biochemistry of murein and cell wall teichoic acids in the genus *Listeria*. Syst. Appl. Microbiol. **5**:360–376.
5. **Gellin, B. G., and C. V. Broome.** 1989. Listeriosis. JAMA **261**:1313–1320.
6. **Kaminsango, K., H. Fujii, H. Okumura, I. Saiki, Y. Araki, Y. Yamamura, and I. Azuma.** 1983. Structural and immunochemical studies of teichoic acid of *Listeria monocytogenes*. J. Biochem. **93**:1401–1409.
7. **Kathariou, S.** 2000. Pathogenesis determinants of *Listeria monocytogenes*, p. 295–314. *In* J. W. Cary, J. E. Linz, and D. Bhatnagar (ed.), Microbial foodborne diseases. Technomics Publishing Co., Inc., Lancaster, Pa.
8. **Kathariou, S., C. Mizumoto, R. D. Allen, A. K. Fok and A. A. Benedict.** 1994. Monoclonal antibodies with a high degree of specificity for *Listeria monocytogenes* serotype 4b. Appl. Environ. Microbiol. **60**:3548–3552.
9. **Lan, Z., F. Fiedler, and S. Kathariou.** 2000. A sheep in wolf's clothing: *Listeria innocua* strains with teichoic acid-associated surface antigens and genes characteristic of *Listeria monocytogenes* serogroup 4. J. Bacteriol. **182**: 6161–6168.
10. **Lei, X.-H., N. Promadej, and S. Kathariou.** 1997. DNA fragments from regions involved in surface antigen expression specifically identify *Listeria monocytogenes* serovar 4 and a subset thereof: cluster IIB (serotypes 4b, 4d, and 4e). Appl. Environ. Microbiol. **63**:1077–1082.
11. **Lu, F., and G. Churchward.** 1995. Tn*916* target DNA sequences bind the C-terminal domain of integrase protein with different affinities that correlate with transposon insertion frequency. J. Bacteriol. **177**:1938–1946.
12. **Morona, R., M. Mavris, A. Fallarino, and P. A. Manning.** 1994. Characterization of the *rfc* region of *Shigella flexneri*. J. Bacteriol. **176**:733–747.
13. **Ochman, H., M. M. Medhora, D. Garza, and D. L. Hartl.** 1990. Amplification of flanking sequences by inverse PCR, p. 219–227. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols. Academic Press, Inc., San Diego, Calif.
14. **Promadej, N., F. Fiedler, P. Cossart, S. Dramsi, and S. Kathariou.** 1999. Cell wall teichoic acid glycosylation in *Listeria monocytogenes* serotype 4b requires *gtcA*, a novel, serotype-specific gene. J. Bacteriol. **181**:418–425.
15. **Schuchat, A., B. Swaminathan, and C. V. Broome.** 1991. Epidemiology of human listeriosis. Clin. Microbiol. Rev. **4**:169–183.
16. **Seeliger, H. P. R., and K. Hoehne.** 1979. Serotypes of *Listeria monocytogenes* and related species. Methods Microbiol. **13**:31–49.
17. **Smith, K., and P. Youngman.** 1992. Use of a new integrational vector to investigate compartment-specific expression of the *Bacillus subtilis spoIIM* gene. Biochimie **74**:705–711.
18. **Uchikawa, K., J. Sekikawa, and I. Azuma.** 1986. Structural studies on teichoic acids in cell walls of several serotypes of *Listeria monocytogenes*. J. Biochem. **99**:315–327.
19. **Ullmann, W. W., and J. A. Cameron.** 1969. Immunochemistry of the cell walls of *Listeria monocytogenes*. J. Bacteriol. **98**:486–493.

*Microbial Pathogenesis* 1997; **22**: 321–329

MICROBIAL PATHOGENESIS

# Detection of the phosphorylcholine epitope in streptococci, *Haemophilus* and pathogenic *Neisseriae* by immunoblotting

**Jan Kolberg,[a]* E. Arne Høiby[b] & Erik Jantzen[a]**

*[a]Department of Vaccinology and [b]Department of Bacteriology, National Institute of Public Health, P.O. Box 4404 Torshov, N-0403 Oslo, Norway*

(Received October 27, 1996; accepted October 31, 1996

The phosphorylcholine (PC) determinant in *Streptococcus pneumoniae* is known to be linked to the cell wall polysaccharides (C-Ps) and to the lipoteichoic acid (LTA) (Forssman antigen) of the plasma membrane. Western blotting with two PC specific murine monoclonal antibodies (MAbs) designated 145,F-2 (IgM) and 147,A-1 (IgA) showed a similar ladder-like pattern for all examined strains of *S. pneumoniae* and *Streptococcus mitis*. Purified antigens run in parallel indicated that this ladder pattern is due to the PC of LTA. Unlike other techniques, Western blotting thus enables the identification of only one of the streptococcal structures carrying the PC epitope. Gram-negative organisms were also examined, and six of 11 *Haemophilus influenzae* strains reacted with the MAbs. For this species, unlike the streptococci, only one fast moving band was detected. Analyses by thin-layer chromatography (TLC) detected the PC epitope in lipopolysaccharide (LPS) fraction from *H. influenzae*. Some strains of the *Neisseriaceae* family were also positive by Western blotting, but TLC and immunostaining did not detect the PC determinant in LPS.



**Key words:** Phosphorylcholine, lipoteichoic acid, lipopolysaccharide, *Streptococcus pneumoniae, Haemophilus influenzae.*

## Introduction

Infections caused by *Streptococcus pneumoniae* still remain a major cause of morbidity and mortality in humans, especially among infants and the elderly. The capsules of the pneumococci are used to classify them in 90 different types [1,2]. Pneumococci have a common cell wall polysaccharide called the C-polysaccharide (C-Ps), and phosphorylcholine (PC) is the immunologically dominant epitope of this antigen. *S. pneumoniae* possesses another PC containing antigen which is chemically related to C-Ps. It is named the Forssman (F) antigen and is a teichoic acid covalently linked to a lipid [3,4]. This lipoteichoic acid (LTA) is a powerful inhibitor of pneumococcal autolysin (N-acetylmuramyl-L-alanine amidase), and its PC residues are involved in the specific interaction with this enzyme [5,6]. When mice are immunized with *S.*

*Author for correspondence.

0882–4010/97/060321+09 $25.00/0 mi960114

*pneumoniae* one of the most prominent antibody responses is to the PC determinant. Several monoclonal antibodies (MAbs) with specificity for PC have consequently been reported. Strains of the genetically related species *Streptococcus mitis* and *Streptococcus oralis* have also been shown to carry epitopes for these MAbs [7–9]. Recently representatives of a wide range of bacterial genera, including *Haemophilus influenzae*, from the mouth and respiratory tract were found to bear the PC determinant [10]. PC epitopes have also been detected in other organisms such as parasites [11–13].

The PC moieties are bound to two different pneumococcal structures. Probing with PC specific MAbs in ELISA or dot blots with heat-killed pneumococci as the antigen most likely detects epitopes in both C-PS and LTA. Methods to differentiate the localization of the PC determinant to one of these pneumococcal structures are therefore of interest. Chemical or affinity purification of LTA from different strains are rather time consuming processes [14]. We here report the application of Western blotting to detect structures carrying the PC determinant in Gram-positive bacteria. We found by this technique that some Gram-negative bacteria also contained PC epitopes, but the pattern of reactivity was different from that seen with Gram-positive bacteria. The structures in *H. influenzae* bearing the PC determinant were by thin-layer chromatography (TLC) found to be lipopolysaccharides (LPS).

## Results

### Specificity analyses of MAbs 145,F-2 and 147,A-1

The binding of the MAbs designated 145,F-2 (IgM) and 147,A-1 (IgA) in ELISA with sonicated pneumococci as coating antigen were inhibited by PC (data not shown). Ethanolamine had no inhibitory effect. These two MAbs reacted by dot blotting with all 76 examined pneumococcal strains representing all the types in the 23-valent polysaccharide vaccine. Dot blotting with other streptococci (Table 1) showed that the MAbs cross-reacted with four out of five *S. mitis* strains whereas three *S. sanguis* strains were non-reactive. Of the examined Gram-negative bacteria six out of 11 *H. influenzae* were positive against the MAbs. No correlation was found with serotypes of the latter organism. None of the 14 strains of *Neissseria meningitis* and likewise none of 13 *Neisseria gonorrhoeae* strains reacted with the MAbs in the dot blot assay.

### Colony blotting

All colonies of the two dot blot positive *H. influenzae* strains (ATCC 31441 and 218/86) were also stained by this technique using MAb 147, A-1. The positive control, a clinical *S. pneumoniae* (233/96) isolate, showed the strongest staining intensity. The non-reactivity of *H. influenzae* strain NCTC 8473 was also confirmed by colony blotting. All examined strains were negative when probed with a MAb of the same isotype but directed against an irrelevant antigen.

### Western blotting

Bacteria were subjected to SDS-PAGE, electrotransferred to nitrocellulose membranes and immunostained with the PC specific MAbs. Twenty of the 76 dot blot examined *S. pneumoniae* strains were randomly selected for analyses by Western blotting, and they showed a ladder pattern (Fig. 1). Of the other examined streptococci also *S. mitis* showed this Western blot pattern (Fig. 1). The *S. mitis* strain 328/87 found negative by dot blotting reacted weakly by Western blotting. A similar ladder pattern was also found for isolated LTA run in parallel, whereas purified C-Ps was non-reactive in this assay, but was positive by dot blotting (Fig.1).

Western blotting with Gram-negative bacteria detected the PC epitope not only in the dot blot positive *H. influenzae* strains, but also in some dot blot negative strains of *N. menigitidis* and *N. gonorrhoeae* (Table 1). For *N. meningitidis* no correlation was found between serogroups and positive staining. The reacting Gram-negative bacteria did not show a ladder pattern by Western blotting. The positive *H. influenzae* strains showed a major, broad, fast moving band (Fig. 1). Using a separating gel containing 15% acrylamide instead of 10%, as in Fig. 1, the *H. influenzae* structures bearing the PC epitope showed some strain variation in their mobilities. They were found in the region between the molecular mass markers of 6.5 and 14.3 kDa. The neisserial reactive bands showed greater strain variations in mol mass than seen for *H.*

Table 1. Dot blot and Western blot staining of the PC specific MAb 147,A-1 with different bacterial strains[a]

| Organisms | No. examined | No. positive | |
|---|---|---|---|
| | | Dot blot | Western blot |
| Streptococci | | | |
| *S. pneumoniae* | 20 | 20[a] | 20 |
| *S. mitis* | 5 | 4 | 5 |
| *S. salivarius* 56/93 | 1 | 0 | 0 |
| *S. bovis* 971/92 | 1 | 0 | 0 |
| *S. sanguis* | 3 | 0 | 0 |
| *S. mutans* 604/92 | 1 | 0 | 0 |
| *S. pyogenes* ATCC 12353 | 1 | 0 | 0 |
| *S. agalactiae* 1010/90 | 1 | 0 | 0 |
| *S. equisimilis* C 74 | 1 | 0 | 0 |
| *S. zooepidemicus* 44/93 | 1 | 0 | 0 |
| *S. suis type* SSI 14636 | 1 | 0 | 0 |
| Other | | | |
| *Haemophilus influenzae*[b] | 11 | 6 | 6 |
| *Neisseria meningitidis*[b] | 14 | 0 | 7 |
| *N. gonorrhoeae*[b] | 13 | 0 | 2 |
| *Enterococcus faecalis* ATCC 19433 | 1 | 0 | 0 |
| *Enterococcus faecium* ATCC 19434 | 1 | 0 | 0 |

[a] Additional 56 pneumococcal strains were tested by dot blotting and found positive.
[b] Representative strains
Positive *H. influenzae*: type b, ATCC 31441; type c, NCTC 8469; non-typeable, HK 223
Weakly positive *H. influenzae*: type a, NCTC 8466
Negative *H. influenzae*: type e, 8472; type f, NCTC 8473; non-typable strains, EF 16552 and HK 387
Weakly positive *N. gonorrhoeae*: 3/96 CIII, SSI

*influenzae*. The bands were found in the region between 14.3 and 21.5 kDa (Fig. 1).

## Specificity analyses of MAb binding to Gram-negative bacteria

Heat-killed *H. influenzae* strain ATCC 31441 was used as coating antigen in ELISA. The binding of MAb 145,F-2 was found to be inhibited by PC, whereas ethanolamine had no effect (data not shown). The PC specific MAbs reacted with *Neisseriae* only by Western blotting. Nitrocellulose strips containing *N. meningitidis* (strain 4/95, group C) or *N. gonorrhoeae* (strain 3/96 CIII) separated by SDS-PAGE were therefore used for inhibition studies. The specificity for PC was confirmed (data not shown). Furthermore, the anti-PC MAb HAS (IgM) [15] showed the same pattern of reactivity. Further, MAbs of isotypes IgA and IgM directed against irrelevant antigens were non-reactive.

## Immunostaining by TLC of LPS and extractable lipids from Gram-negative bacteria

Lipids and LPS were extracted from MAb 147, A-1 positive and negative strains of *H. influenzae* and *N. meningitidis*. These two partially purified fractions and the residual protein fraction were in prelimminary dot blot experiments spotted to TLC plates and immunostained with the PC specific MAb 147,A-1. The *N. meningitidis* strain 1030/95, which by Western blotting was found to carry PC epitopes, showed a very weak immunostaining only of the protein fraction. The strain ATCC 31441 of *H. influenzae* revealed the strongest immunostaining of the partially purified LPS, but also some staining of both the protein and the extractable lipid fractions.

LPS showed the same TLC mobility of the orcinol-positive component of strain ATCC 31441 and the component that immunostained with MAb 147,A-1 (Fig. 2). LPS from the dot

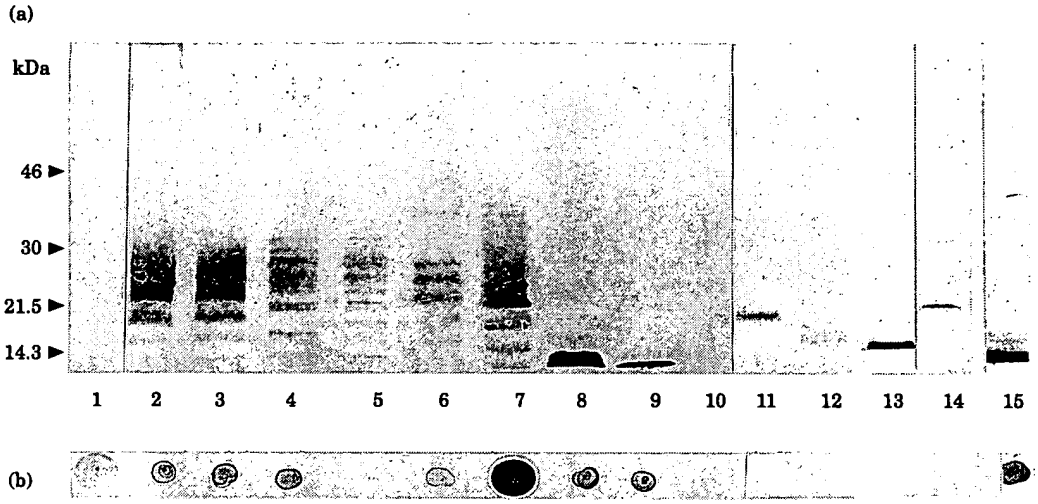



**Figure 1.** Western blot (a) and dot blot (b) of bacteria and purified pneumococcal antigens probed with the PC specific MAb 147,A-1 (samples 1–14) and the LPS specific MAb 9-2-L379 (sample 15). The MAbs 147,A-1 and 9-2-L379 were used as ascitic fluids diluted 1:2000 and 1:200000, respectively. The amounts of purified pneumococcal C-Ps and F-antigen (LTA) used were 1.5 μg in both assays. The bacterial samples for Western blotting contained about 12 μg protein, whereas the bacterial samples for dot blotting contained about 0.4 μg. Lane 1 and dot 1: C-PS; lanes 2–3; two clinical isolates of *S. pneumoniae*; lanes 4–6; three clinical isolates of *S. mitis*; lane 7: purified F-antigen (LTA); lane 8: *H. influenzae* ATCC 31441; lane 9: *H. influenzae* NCTC 8469; lane 10: *H. influenzae* EF 16552; lane 11: *N. meningitidis* 458/95; lane 12: *N. meningitidis* 61/95; lane 13: *N. meningitidis* 4/95; lane 14: *N. gonorrhoeae* 3/95; lane 15: *N. meningitidis* 458/95.

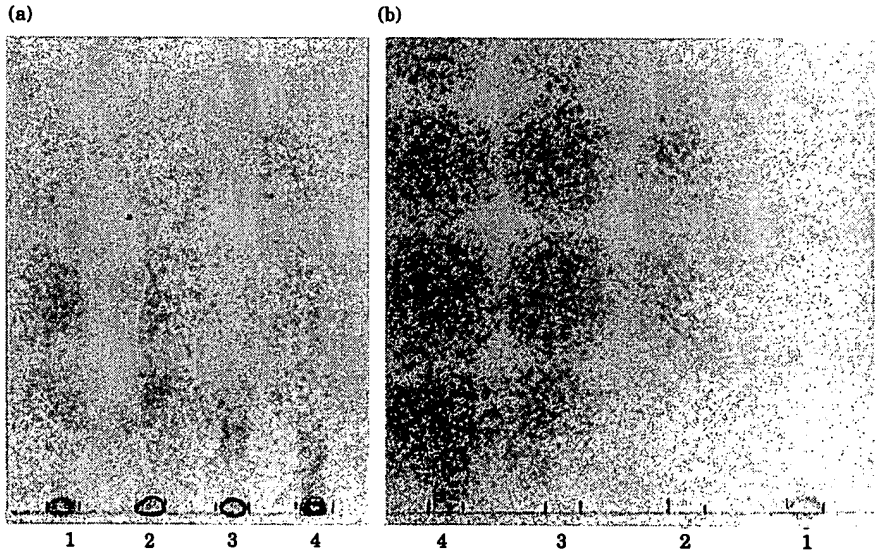



**Figure 2.** Thin-layer chromatography of LPS from *H. influenzae*, *N. meningitidis* and pneumococcal F-antigen (LTA). (a) Stained with orcinol, (b) immunostained using MAb 147,A-1 (ascitic fluid diluted 1:200). Sample 1: pneumococcal F-antigen (LTA); sample 2: *N. meningitidis* 1030/95; sample 3: *H. influenzae* NCTC 8473; sample 4: *H. influenzae* ATCC 31441.

blot and Western blot non-reacting *H. influenzae* strain NCTC was also negative in this assay. The positive control with pneumococcal LTA (F-antigen) did not move from the application site in this system (Fig. 2). On the other hand, LPS of the Western blot positive *N. meningitidis* strain 1030/95 did not show immunostaining with the MAb (Fig. 2). The immunostaining in this TLC system was considerably weaker compared to Western blotting, although the MAb concentration was increased 10 times.

TLC of the extractable lipid fractions followed by immunostaining showed only a weak staining on the application sites for strain ATCC 31441 and pneumococcal LTA (results not shown). Staining with $CuSO_4$/phosphoric acid showed that the lipids had migrated whereas LTA had not. In contrast to immunostaining of LPS after TLC, the lipids from the three examined Gram-negative bacteria showed a brownish, unspecific staining of spots with the same mobility as those visualized with $CuSO_4$/phosphoric acid. However, the brownish colour was clearly different from the red coloured spots considered as positive reactions.

## Discussion

All the 76 examined pneumococcal strains showed strong dot blot reactions with two selected MAbs (145,F-2; 147,A-1) with specificity for the immunodominant PC group of pneumococcal C-Ps. This expression of the PC epitope is consistent with other studies using MAbs or antisera against C-Ps [7–9]. The *S. suis* strain SSI 14636 reported not to contain C-Ps [7] was also non-reactive in our assays. We found that the 3 examined strains of *S. sanguis* did not carry the PC epitope, whereas others have found 1 out of 4 to be positive [7].

About 90% of the choline in *S. pneumoniae* is a constituent of the C-Ps, and most of the remainder is located within LTA [3], another common pneumococcal antigen. LTA is a lipoteichoic acid, i.e. a teichoic acid covalently linked to lipids in the plasma membrane. Teichoic acid and lipoteichoic acid of *S. pneumoniae* possess identical chain structures [4].

Purified pneumococcal C-Ps immunostained with the PC specific MAbs by dot blotting, but was non-reactive by Western blotting (Fig. 1). The reason for this is most likely that these macromolecules do not move into the PAGE separation gel. C-Ps is covalently linked to peptidoglycan [16] and will therefore not be dissociated by SDS treatment. Purified pneumococcal F antigen (LTA) was positive by both methods and showed a ladder pattern with the PC specific MAbs in Western blotting. This pattern, found for all examined *S. pneumoniae* isolates, is most likely due to the reported heterogeneity in chain length, which may vary between two and eight repeating units, as well as the variation in the fatty acid composition of LTA [17].

*S. mitis* has been reported to carry the PC epitope [7–9]. We found by Western blotting a ladder pattern of *S. mitis* similar to that of *S. pneumoniae*; indicating the presence of LTA-like structures. Pneumococcal LTA differs from most known LTA by containing ribitol phosphate instead of glycerol phosphate [17]. *S. mitis* also contains ribitol teichoic acids and choline. [18]. From our studies we cannot say whether *S. mitis* like *S. pneumoniae* also has the PC determinant in C-Ps or not.

We found by dot blotting, colony blotting and Western blotting that some, but not all, strains of the Gram-negative organism *H. influenzae* expressed the PC epitope. This is in accordance with the recent report by Gillespie *et al.* [10] using capture ELISA to detect the PC epitope in 29 out of 100 *H. influenzae* strains.

Unlike the streptococci, *H. influenzae* in Western blotting showed one broad, fast moving band. TLC analyses of the structures bearing this epitope showed that it was located in the LPS fraction. LPS like LTA is a group of glycolipids that are surface exposed. A surface expression of the PC determinant is not in accordance with the reported localization to the plasma membrane by using immunogold labelling of ultra-thin sections of *H. influenzae* [10].

The PC bearing structures in some strains of *N. meningitis* and *N. gonorrhoeae* do not seem to be LPS because the reacting bands seen by Western blotting were of much lower mobility than that seen with the LPS specific MAb (Fig. 1). Furthermore, we could not detect the PC determinant by TLC of LPS from one examined Western blot positive strain of *N. meningitis*. Only the residual fractions after extraction of lipids and LPS showed a weak immunostaining by dot blotting. We therefore cannot conclude from our experiments the nature of the neisserial structures that contain the PC epitopes. The structures do not seem to be surface exposed because they could not be detected in the dot

blot assay. However, this observation may also be related to the relative amount of antigen used by the two methods. Measured as bacterial proteins, the amount was about 30 times higher for the Western blot technique compared to the dot blot assay. It should also be noted that one weakly Western blot positive *S. mitis* strain was non-reactive by dot blotting (Fig. 1). An intracellular localization of the PC determinant could explain why other workers have tested four *N. meningitidis* with negative results in capture ELISA using whole cells [8]. One strain of *N. meningitidis* was found negative in ELISA with disrupted cells [10]. It is also a possibilty that these strains did not carry the PC determinant. Interestingly, specific anti-PC immune responses in mice induced by one strain of *N. meningitis* group B has been detected with a plaque forming cell assay [19,20].

Virulent pneumococci have been shown to invade eucaryotic cells by attachment of bacterial PC to the G-protein coupled platelet activating factor (PAF) [21], a biologically active phospholipid. Our findings raise the question whether the PC determinant found in some strains of *H. influenzae* also contributes in the adherence and invasion of this species by using the PAF receptor and thus may be a virulence factor.

## Materials and methods

### Bacterial strains

The bacteria examined are given in Table 1. Most of the strains were human clinical isolates from our institute, but some were from the American Type Culture Collection (ATCC), Rockville, MD, U.S.A. or the National Collection of Type Cultures (NCTC), Central Public Health Laboratory, London, England. Statens Seruminstitut (SSI), Copenhagen, Denmark, gave us *S. suis* type 8 (strain 14636) and seven reference strains of *N. gonorrhoeae*. *H. influenzae* strains EF 16552, HK 223 and HK 387 were obtained from Dr. Mogens Kilian, Institute of Medical Microbiology, University of Aarhus, Aarhus, Denmark. *S. sanguis* was classified by conventional tests according to Coykendall [22] and the API 20 Strep system from bioMerieux, Marcy-l'Etoilé, France.

### Pneumococcal components and MAbs

C-Ps, F-antigen (LTA) and the PC specific MAb designated (HAS) [15] were kindly supplied by Dr. Jørgen Henrichsen, SSI, Copenhagen, Denmark. The MAb 9-2-L3,7,9 with specificity for meningococcal LPS immunotypes L3,L7, and L9 [23] was obtained from Dr. E. E. Moran, Walter Reed Army Institute of Research, Washington, DC, U.S.A.

### Production of MAbs

Heat-inactivated (30 min at 56°C) and sonicated pneumococcal strains of types 8 and 4 were used as antigens. The former antigen resulted in MAb 145,F-2 and the latter in MAb 147,A-1.

Six-week-old BALB/c mice were immunized intraperitoneally with bacterial suspensions containing 50 µg protein in 0.25 ml phosphate buffered saline (PBS) mixed with 0.25 ml Freund's incomplete adjuvant, followed by a booster injection 2 weeks later with the same mixture. The fusions were performed 4 months later. MAb 145,F-2 was generated after intraperitonal injection of the above-mentioned antigen in Freund's incomplete adjuvant on day 7 followed by the same route of immunization with the antigen in PBS on days 4 and 3 before fusion. MAb 147,A-1 was produced after intraperitonal injection with the antigen in Freund's incomplete adjuvant on day 5. The immunization route was then changed by giving the mouse intravenously an injection of the antigen in PBS on day 5. Fusions were performed by standard techniques.

Cell culture supernatants were screened by ELISA (see below) against the pneumococcal strain used for immunization and against C-Ps. Those positive against both antigens were expanded and retested in ELISA with an irrelevant antigen (ovalbumin) to exclude unspecifically binding antibodies. MAb binding to C-Ps was then analysed for inhibition by PC (see below). Selected hybrids were cloned by limiting dilution. Isotyping of MAb in cell culture medium was performed in ELISA (see below) using a kit (93-6550) from Zymed Lab. Inc., South San Francisco, CA, U.S.A.

### Protein assay

To determine protein concentrations, aliquots of bacteria were dissolved in 0.5 M NaOH. Lowry's method [24] was used with bovine serum albumin as standard.

## ELISA measurements

Flat-bottomed microtitre plates (MaxiSorp™, Nunc A/S, Roskilde, Denmark) were coated with heat-killed and sonicated bacteria, 25 μg protein/ml in PBS, 100 μl per well. The C-Ps antigen was coated with 1 μg/ml in PBS. Washing, incubations with antibodies, enzyme and substrate were performed as previously described [25].

## Inhibition analyses

Ascitic fluids or cell culture media containing MAbs were titrated in microtitre plates with pneumococcal strain 11/89 (type 14) or *H. influenzae* ATCC 31441 as coating antigens to find dilutions giving an OD of about 1.0. MAbs diluted to these concentrations were incubated in tubes for 2h at 37°C with PC or ethanolamine in the concentration range 2 to 320 mM. One aliquot was incubated only with buffer (0.05 M Tris-HCl in 0.15 M NaCl, pH 7.4). Samples (100 μl) were then added to the microtitre plates and they were then incubated for 2h at 37°C, followed by alkaline phosphatase conjugated antibody and substrate, as described above.

Samples of MAb with various concentrations of inhibitors were also incubated with nitrocellulose membrane strips containing bacterial proteins separated by SDS-PAGE. The strips had first been blocked with 3% bovine serum albumin (BSA) in PBS. Incubations with peroxidase-conjugated antibodies and substrate were done, as described below. Some experiments were also performed using the PC specific MAb HAS [15] as control.

## SDS-PAGE and immunoblotting

The bacterial suspensions in PBS were boiled for 5 min with sample buffer containing 2-mercaptoethanol. Samples with 12 μg protein were applied into each well formed by a 10-tooth comb with Bio-Rad Mini-Protean Slab Cell apparatus. SDS-PAGE was performed with stacking and separating gels containing 4 and 12% acrylamide, respectively. Some experiments were also performed with 15% in the latter gel. The antigens were electrotransferred to nitrocellulose membranes (pore size 0.2 μm). To prevent non-specific binding of proteins, the membranes were incubated for 30 min in a blocking buffer consisting of 3% BSA in PBS. The primary antibodies were used as ascitic fluids diluted 1:2000, and as secondary antibody was used peroxidase-conjugated rabbit immunoglobulins against mouse immunoglobulins (dilution 1:1000, Dako A/S, Glostrup, Denmark). The immunostaining was performed with 3-amino-9-ethylcarbazole and $H_2O_2$ in sodium acetate buffer (pH 5.0). The Rainbow protein molecular weight markers from Amersham International plc, Buckinghamshire, U.K., were used.

## Dot blot assay

Heat-treated (56°C, 30 min) bacteria from stationary phase growth were spotted to nitrocellulose membranes, as previously described [25]. The primary antibodies were used as cell culture medium diluted 1:2 and 1:100. Bound MAbs were detected with peroxidase-conjugated antibodies (see above).

## Colony blotting

A bacterial suspension (12.5 μl) diluted to contain about 100 cfus of bacteria for each strain tested was prepared from overnight growth on chocolate agar. Four different strains were inoculated onto the same chocolate agar plate, each on one quarter of it. Each inoculum was separated by ditches cut in the agar with a scalpel. The plates were grown overnight at 37°C in 5% $CO_2$. Well separated single colonies of each of the four strains on one single plate were blotted directly onto one nitrocellulose filter. After blocking in 3% BSA in PBS, the filters were probed for 90 min with MAbs used as ascitic fluid diluted 1:200. The washed filters were incubated with secondary antibody and developed as previously described for dot blotting [25].

## Extraction of lipids and LPS

Four strains were selected for analyses of structures bearing the PC epitope, two with the epitope (*H. influenzae* ATCC 31441, *N. meningitidis* 1030/95) and two without (*H. influenzae* NCTC 8473, *N. meningitidis* 44/76). The bacteria were incubated overnight at 37°C on standard plates, with GK chocolate agar for *Haemophilus* and Kellogg medium for *Neisseria*. The bacteria from

each plate were harvested in 2ml PBS. The samples from each strain were pooled, heat-treated for 40 min at 56°C followed by centrifugation (15000 rpm, 20 min). The bacteria were washed twice in PBS.

Lipids were first extracted from the pellets by the method of Kates [26]. Briefly, the wet cells were transformed to glass tubes (8ml) with teflon-lined screw caps and homogenized by a glass rod before addition of 3.75ml methanol-chloroform (2:1). The mixture was shaken occasionally for 3h at 25°C. After centrifugation for 10 min at 2000 rpm, the residue was re-extracted with 4.75ml methanol–chloroform–water (2:1:0.8). The combined supernatants were added 2.5ml chloroform and 2.5ml water, shaken and centrifuged. The bottom phase was collected by use of a Pasteur pipette; and taken to dryness by nitrogen-bubbling, dried by repeated evaporation after addition of 1ml dry ethanol. Residual material was dissolved in 0.5ml chloroform–methanol (1:1).

LPS extraction was performed by the hot phenol procedure [27]. The residue after lipid extraction was dried by nitrogen before addition of 1.5ml 90 % phenol (preheated to 68°C) and 1.5ml distilled water (68°C). The mixture was kept at 68°C with occasional shaking on a whirlimixer. After cooling in ice and centrifugation for 20 min at 2000 rpm and 4°C, the top water phase was transferred to another tube and bottom phenol phase re-extracted with 1.5ml distilled water (68°C). The combined water phases were then dialysed against distilled water and thereafter freeze-dried.

### Thin-layer chromatography (TLC)

High performance silic acid TLC plates (Alsheets, Merck AG, Darmstadt, Germany) were used. The lipid fractions were developed twice with a solvent mixture of chloroform–methanol–acetic acid–water (85:15:10:3.5). Spots were visualized by dipping in an acidic $CuSO_4$/phosporic acid solution as described [28] or immunostaining as previously reported [29]. Briefly, one half of the chromatogram was incubated with MAb 147,A-1 (ascitic fluid diluted 1:100) followed by alkaline phosphatase-conjugated rabbit antibodies to mouse immunoglobulins (dilution 1:1000, Dako A/S, Glostrup, Denmark). Antibody reactions were visualized by adding Fast Red TR salt (Serva, Heidelberg, Germany) and Naphthol AS-MX phosphate.

The LPS fractions were developed twice by a solvent mixture of isopropanol–water–chloroform–ammonia–triethylamine (120:60:16:4:1), and spots were visualized by dipping in 0.1% orcinol in 30% aqueous methanol or by incubation with MAb 147,A-1 (ascitic fluid diluted 1:200), as described above.

## Acknowledgements

Elisabeth Fritzsønn, Anne Lise Heistø, Gro Lermark, Torunn Marigård and Gunnhild Rødal provided excellent technical assistance.

## References

1 Lund E, Henrichsen J. Laboratory diagnosis, serology and epidemiology of *Streptococcus pneumoniae*. In: Bergan T, Norris JR, eds. *Methods in Microbiology*. London: Academic Press 1978; 241–62.
2 Henrichsen J. Six newly recognized types of *Streptococcus pneumoniae*. *J Clin Microbiol* 1995; **33**: 2759–62.
3 Briles EB, Tomasz A. Pneumococcal Forssman antigen. A choline-containing lipoteichoic acid. *J Biol Chem* 1973; 248: 6394–7.
4 Fischer W, Behr T, Hartmann R, Peter-Katalinić J, Egge H. Teichoic acid and lipoteichoic acid of *Streptococcus pneumoniae* possess identical chain structures. A reinvestigation of teichoid acids (C polysaccharide). *Eur J Biochem* 1993; **215**: 851–7.
5 Briese T, Hakenbeck R. Interaction between choline and the N-acetyl-L-alanine amidase of *Streptococcus pneumoniae*. In: Hackenbech R, Holtjeand JV, Labischinski H, eds. *The Target of Penicillin. The Murein Sacculus of Bacterial Cell Walls; Architecture, and Growth*. Berlin: Walther de Gruyter, 1983: 173–8.
6 Horne D, Tomasz A. Pneumococcal Forssman antigen: enrichment in mesosomal membranes and specific binding to the autolytic enzyme of *Streptococcus pneumoniae*. *J Bact* 1985; **161**: 18–24.
7 Sørensen UBS, Henrichsen J. Cross-reactions between pneumococci and other streptococci due to C polysaccharide and F antigen. *J Clin Microbiol* 1987; **25**: 1854–9.
8 Sjögren AM, Holmberg H, Krook A. Etiologic diagnosis of pneumonia by antigen detection: crossreactions between pneumococcal C-polysaccharide and oral microorganisms. *Diagn Microbiol Infect Dis* 1987; **6**: 239–48.
9 Gillespie SH, McWhinney PHM, Patel S, Raynes JG, McAdam KPWJ, Whiley RA, Hardie JM. Species of alpha-hemolytic streptococci possessing a C-polysaccharide phosphorylcholine-containing antigen. *Infect Immun* 1993; **61**: 3076–7.
10 Gillespie SH, Ainscough S, Dickens A, Lewin J. Phosphorylcholine-containing antigens in bacteria from the

mouth and respiratory tract. *J Med Microbiol* 1996; **44**: 35–40.
11 Sugane K, Oshima, T. Activation of complement in C-reactive protein positive sera by phosphorylcholine-bearing component isolated from parasite extract. *Parasite Immunol* 1983; **5**: 385–95.
12 Lim, PL, Choy, WF. A thymus-independent (type 1) phosphorylcholine antigen isolated from *Trichinella spiralis* protects mice against pneumococcal infections. *Immunol* 1990; **69**: 443–8.
13 Burkot, TR, Kwan-Lim, GE, Maizels, RM. A novel 95-kilodalton antigen of *Wuchereria bancrofti* infective larvae identified by species-specific monoclonal antibodies. *Infect Immun* 1996; **64**: 485–8.
14 Amoroso, A, Washington, BA, Gutkind, G, Doyle, RJ. An affinity chromatografic method for the preparation of bacterial lipoteichoic acids. *J Microbiol Meth* 1996; **25**: 19–22.
15 Sørensen UBS, Agger R, Bennedsen J, Henrichsen J. Phosphorylcholine determinants in six pneumococcal capsular polysaccharides detected by monoclonal antibody. *Infect Immun* 1984; **43**: 876–8.
16 Fischer H, Tomasz A. Peptidoglycan cross-linking and teichoic acid attachment in *Streptococcus pneumoniae*. *J Bact* 1985; **163**: 46–54.
17 Behr T, Fischer W, Peter-Katalinić J, Egge H. The structure of pneumococcal lipoteichoic acid. Improved preparation, chemical and mass spectrometric studies. *Eur J Biochem* 1992; **207**: 1063–75.
18 Kilpper-Bälz R, Wenzig P, Schleifer KH. Molecular relationships and classification of some viridans streptococci as *Streptococcus oralis* and emended description of *Streptococcus oralis* (Brigde and Sneath 1982). *Int J Syst Bact* 1985; **35**: 482–8.
19 Faro J, Seoane R, Puentes E, Ubeira FM, Regueiro BJ. Immunoresponses to *Neisseria meningitidis* epitopes: primary versus secondary antiphosphorylcholine responses. *Infect Immun* 1985; **48**: 428–32.
20 Faro J, Seoane R, Lareo I, Eiras A, Couceiro J, Regueiro BJ. Immunoresponses to *Neisseria meningitidis* epitopes: immunodulation by meningococcus B acts on more than one meningococcal surface epitope. *Med Microbiol Immunol* 1987; **176**: 131–41.
21 Cundell DR, Gerard NP, Gerard C, Idanpaan-Heikkila I, Tuomanen EI. *Streptococcus pneumoniae* anchor to activated human cells by the receptor for platelet-activating factor. Nature 1995; **377**, 435–8.
22 Coykendall, AL. Classification and identification of the viridans streptococci. *Clin Microbiol Rev* 1989; **2**: 315–28.
23 Moran EE, Brandt BL, Zollinger WD. Expression of the L8 lipopolysaccharide determinant increases the sensitivity of *Neisseria meningitidis* to bactericidal activity. *Infect Immun* 1994; **62**: 5290–5.
24 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265–75.
25 Kolberg J, Aaberge IS, Jantzen E, Løvik M, Lermark G, Steen M. Murine monoclonal antibodies against pneumococcal capsular polysaccharide types 4, 8, 23F and 19A/19F. *APMIS* 1992; **100**: 91–4.
26 Kates, M. Techniques of lipidology: isolation, analysis and identification of lipids. In: Work TS, ed. *Techniques in Biochemistry and Molecular Biology*. Amsterdam: North-Holland Publishing Company, 1972: 351.
27 Westphal O, Lüderitz O, Bister R. Über die extraction von bacterien mit phenol/wasser. *Zeitschr Naturforsch* 1975, **7b**: 148–55.
28 Aase B, Jantzen E, Bryn K, Ormerod J. Lipids of heliobacteria are characterized by a high proportion of monoenoic fatty acids with variable double bound positions. *Photosynthesis Res* 1994; **41**: 67–74.
29 Andersen SR, Bryn K, Thorseng K, Jantzen E. Heterogeneity of lipopolysaccharides of *Neisseria meningitidis* revealed by thin-layer chromatography combined with monoclonal antibodies. *J Microbiol Meth* 1996; **25**: 187–94.

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1988, p. 1635–1640
0095-1137/88/091635-06$02.00/0

Vol. 26, No. 9

# Cross-Reactive Monoclonal Antibodies for Diagnosis of Pneumococcal Meningitis

W. DOUGLAS WALTMAN II,[1]* BARRY GRAY,[2] LARRY S. McDANIEL,[1] AND DAVID E. BRILES[1,2]

*Departments of Microbiology[1] and Pediatrics,[2] University of Alabama at Birmingham, Birmingham, Alabama 35294*

Received 21 January 1988/Accepted 19 May 1988

**A diagnostic test for the detection of *Streptococcus pneumoniae* meningitis was developed using monoclonal antibodies (MAbs) to phosphocholine (PC) and non-PC determinants of pneumococcal teichoic acids. These MAbs do not recognize other bacteria that commonly cause meningitis. By using a dot blot assay, these MAbs were compared with a polyvalent pneumococcal capsular omniserum and an antiserum made to whole cells for their ability to detect pneumococci in infected spinal fluids. An immunoglobulin M (IgM) anti-PC antibody gave a positive reaction with 16 of 22 (73%) pneumococcal culture-positive spinal fluids. One false-positive result out of 45 pneumococcal culture-negative spinal fluids was also observed. D3114/63, an IgM MAb to non-PC determinants of teichoic acids, detected 15 of 22 of the pneumococcal culture-positive spinal fluids with one false-positive result. IgG2b and IgG3 anti-PC MAbs were less efficient than the IgM anti-PC MAb at detecting pneumococci in spinal fluids. Like the IgM anti-PC MAb, omniserum detected 73% of the culture-positive pneumococcal spinal fluids, with one false-positive result. The use of anti-PC or D3114/63 MAbs instead of a pooled serum such as omniserum has several advantages: (i) use of a single cross-reactive antibody rather than 83 pooled antibodies; (ii) possibility of a higher concentration of reactive antibody, which may increase the sensitivity of the test; (iii) a standardized antibody preparation; (iv) ease of preparation of the antibody; and (v) less expense.**

The rapid diagnosis of pneumococcal infections, in particular meningitis, is of critical importance. Since the highest incidence of pneumococcal meningitis occurs in children less than 2 years of age and because of the high case fatality rate of this infection (7, 10), a rapid, reliable diagnostic test would greatly improve the prognosis of patients with this disease. Several assay methods have been studied to determine their effectiveness in diagnosing pneumococcal meningitis, such as counterimmunoelectrophoresis (3, 16, 17), latex agglutination (16, 17), and coagglutination (4, 14, 16, 17). The sensitivities of the methods vary among studies, but in general counterimmunoelectrophoresis is the least sensitive, followed by coagglutination and latex agglutination.

A major difficulty in devising a diagnostic assay for pneumococcal meningitis is the multiplicity of capsular serotypes of *Streptococcus pneumoniae*. Most of the diagnostic assays developed thus far have used a pooled anticapsular serum, commonly omniserum, for the detecting antibody. This type of pooled antiserum suffers from at least two disadvantages. First, because so many different antibodies are included in the pool, the antibody concentration of any one capsular serotype may not be high enough to detect low concentrations of antigen. Second, cross-reactions, in particular with alpha streptococci, have been found to be associated with this antiserum (9). An alternative approach to using a pooled antiserum would be the use of a single antibody which would react with an antigenic determinant common to all pneumococci. Antibodies to the phosphocholine (PC) determinant of the pneumococcal cell wall and to a determinant in the pneumococcal teichoic acid recognized by the monoclonal antibody (MAb) D3114/63 have been shown to react with all pneumococci tested and do not react with the other streptococci tested (13).

The present study was undertaken in part to determine the usefulness of anti-PC and D3114/63 MAbs in the diagnosis of pneumococcal meningitis. The study also allows the comparison of the usefulness of MAbs in different types of assays, as well as comparisons of the relative sensitivities of assays with immunoglobulin M (IgM) and IgG MAbs. The results obtained from comparisons with IgG and IgM anti-PC MAbs are particularly valuable because these particular antibodies are known to have essentially identical idiotypes and binding specificities (1). Thus any differences observed can be assumed to be caused by differences in isotype rather than differences in binding site.

## MATERIALS AND METHODS

**Spinal fluids.** Sixty-one culture-positive pediatric spinal fluids were collected. The culture-positive spinal fluids consisted of the following bacterial species: *S. pneumoniae* (22 fluids), *Haemophilus influenzae* type b (20 fluids), *Neisseria meningitidis* (7 fluids), group B streptococci (7 fluids), *Listeria monocytogenes* (2 fluids), viridans group streptococci (1 fluid), *Staphylococcus aureus* (1 fluid), and *Klebsiella* sp. (1 fluid). The 22 spinal fluids positive for pneumococci contained the following capsular types: type 3 (two isolates), type 6 (six isolates), type 8 (one isolate), type 10 (one isolate), type 12 (one isolate), type 14 (two isolates), type 18 (one isolate), type 19 (three isolates), type 23 (three isolates), type 33 (one isolate), and type 35 (one isolate). Six culture-negative spinal fluids were also obtained and were included as controls. Each spinal fluid was stored at −70°C. Before screening, each spinal fluid was coded and tested in a blind format.

**Antibodies.** The MAbs and antisera used in this study are listed in Table 1. The production of all of these antibodies has been described in earlier publications (1, 2, 6, 13). Quantitation of the anti-PC antibodies was by Farr assay with $^{14}$C-labeled PC chloride. The number of binding sites in

* Corresponding author.

TABLE 1. Description of the antibodies used to screen spinal fluid samples

| Antibody | Antibody type | Isotype | Antibody concn (mg/ml) | Epitope | Reference |
|---|---|---|---|---|---|
| 140.1C2[a] | MAb | IgG2b(κ) | 1.3 | PC | 2 |
| 59.6C5[a] | MAb | IgG3(κ) | 0.164 | PC | 2 |
| HPCM2[b] | MAb | IgM(κ) | 2.0 | PC | 6 |
| D3114/63 | MAb | IgM(κ) | ? (ascites fluid) | Teichoic acid | 13 |
| Anti-R36A | Polyvalent | | ? (serum) | Whole cell | |
| Omniserum | Polyvalent pool | | ? (serum) | Capsule | 11, 12 |

[a] Obtained from Lathum Claflin, University of Michigan, Ann Arbor.
[b] Obtained from Patricia Gearhart, Johns Hopkins University, Baltimore.

each sample was estimated by extrapolation to saturating PC. The amount of each antibody was calculated based on the known molecular weight and number of binding sites of each isotype. The MAbs were produced as ascites fluid in X-linked immunodeficient (*xid*) (CBA/N × BALB/cJ)$F_1$ mice and diluted 1:40 in phosphate-buffered saline (PBS; pH 7.2). A polyvalent serum was included which was prepared by immunizing rabbits with the heat-killed pneumococcal strain R36A (a rough derivative of the serotype 2 strain D39). Omniserum obtained from Statens Serum Institute (Copenhagen, Denmark), which contains antibodies to all known pneumococcal capsular serotypes, was also included for comparison. A 1:50 dilution in PBS of both the anti-R36A and omniserum was used for testing.

**Reactivity of MAbs with pneumococci and other bacteria.** *S. pneumoniae* and other gram-positive and gram-negative bacteria were collected and screened for reactivity to anti-PC and D3114/63 MAbs by using the colony blot and lysate blot techniques of McDaniel et al. (13) and Waltman et al. (W. D. Waltman II, L. S. McDaniel, B. Andersson, L. Bland, B. M. Gray, C. S. Eden, and D. E. Briles, Microb. Pathogen., in press). The colony blot procedure is very similar to the dot blot assay, except that blots of bacterial colonies rather than bacterial lysates are tested.

**Dot blot assay.** Fifteen microliters of the respective spinal fluids or standardizing solutions (i.e., live or heat-killed pneumococci, pneumococcal lysates, or PC-bovine serum albumin [BSA]) were spotted onto sterile nitrocellulose membranes, one membrane for each antibody and a BSA control, allowed to air dry, and blocked for 30 min with a solution of 1% BSA in PBS. The membrane was washed twice in PBS containing 0.05% Tween 20 (PBST) and incubated with the respective antibody at room temperature for 3 h on a rocking platform. After three washes in PBST, a 1:750 dilution (in PBST) of alkaline phosphatase-labeled goat anti-mouse immunoglobulin conjugate (0.2 mg/ml; Southern Biotechnics Associates, Birmingham, Ala.) was incubated for 3 h with the membranes probed with the anti-PC and D3114/63 antibodies. A 1:750 dilution of alkaline phosphatase-labeled goat anti-rabbit immunoglobulin conjugate (0.2 mg/ml; Southern Biotechnics Associates) was incubated for 3 h with the membranes probed with anti-R36A and omniserum antibodies. The membranes were washed three times in PBST, and a solution of 0.5 mg of 5-bromo-4-chloro-3-indolyl phosphate (Sigma Chemical Co., St. Louis, Mo.) per ml in 1 M Tris (pH 8.8) was incubated with the membranes for approximately 60 min at room temperature with gentle rocking. The reaction was stopped by rinsing the membranes with distilled water. Positive reactions were visualized by blue staining areas on the sample spot.

**ELISA.** An enzyme-linked immunosorbent assay (ELISA) was set up to determine the level of antigen detected by each of the antibodies and to determine the feasibility of making a more rapid diagnostic test. This procedure consisted of absorbing either 100 μl of a solution of $10^8$ heat-killed R36A cells per ml (centrifuged in a microdilution plate for 15 min at 1,500 rpm) or 100 μl of 10 μg of PC-BSA per ml (incubated overnight at 4°C) onto a microdilution plate followed by blocking with 1% BSA for 1 h. The subsequent incubations with the antibody and enzyme-labeled conjugate followed standard methods and were identical with those of the dot blot assay, except that incubation was at 37°C. The substrate, a solution of 1 mg of *p*-nitrophenyl phosphate (Sigma) per ml in diethanolamine buffer (pH 9.6) was added after the incubation with the enzyme-labeled second antibody and allowed to react for 30 min at room temperature. The reaction was stopped by adding 50 μl of 3 N NaOH, and the plate was read with a Titertek Multiskan (Flow Laboratories, McLean, Va.) microdilution plate reader at 405 nm. Values greater than twice the background level were considered positive.

To determine the feasibility of making a more rapid diagnostic assay, the ELISA parameters outlined above were modified to determine the optimal conditions necessary to run the assay in the shortest possible time. The titers of antigen, antibody, and conjugate were determined, and the incubation times and temperature were altered.

**Determination of assay sensitivity.** A serotype 14 strain, BG-215, was grown in 150 ml of Todd-Hewitt broth supplemented with 0.5% yeast extract for 12 h at 37°C. The culture was divided into three parts; 50 ml was used as whole live bacteria, 50 ml was heat killed at 60°C for 30 min, and 50 ml was pelleted and lysed with a lysing buffer (0.1% sodium deoxycholate, 0.01% sodium dodecyl sulfate, 0.15 M sodium citrate). The number of bacteria present was determined by standard plate count on blood agar for the live and heat-killed (before heating) samples, and a protein concentration (Bio-Rad Laboratories, Richmond, Calif.) was measured for the lysate. Tenfold serial dilutions of each preparation were made in PBS, and 15 μl was spotted onto a nitrocellulose membrane and assayed as described above. As a control, PC-BSA was serially diluted in PBS, and 15 μl was added to the membrane and assayed. Samples of 100 μl of the same preparations were tested in an ELISA format, as outlined above, by substituting them for the heat-killed pneumococci as the absorbed antigen.

**ELISA inhibition assay.** One hundred microliters containing $10^8$ heat-killed pneumococcal strain R36A cells was absorbed onto a microdilution plate as described above. Fifty microliters of the individual spinal fluids was serially (twofold) diluted in a microdilution plate, 50 μl of a 1:1,000 dilution of HPCM2, D3114/63, anti-R36A or omniserum was added to each well, and plates were incubated for 3 h at 37°C. Subsequent steps, washing in PBST, incubation with alkaline phosphatase-labeled anti-immunoglobulin, washing in PBST, and incubation with the substrate, followed the

TABLE 2. Accuracy of the dot blot assay for diagnosing pneumococcal meningitis from spinal fluid samples[a]

| Antibody | No. with pneumococcal culture | | % Sensitivity[b] | % Specificity[c] | % Agreement[d] |
|---|---|---|---|---|---|
| | + | − | | | |
| HPCM2 + | 16 | 1 | 72.7 | 97.8 | 89.6 |
| − | 6 | 44 | | | |
| D3114/63 + | 15 | 1 | 68.2 | 97.8 | 88.0 |
| − | − | 7 | 44 | | |
| Omniserum + | 16 | 1 | 72.7 | 97.8 | 89.6 |
| − | 6 | 44 | | | |
| Anti-R36A + | 19 | 26 | 86.4 | 42.2 | 56.7 |
| − | 3 | 19 | | | |

[a] MAb HPCM2 was diluted to 50 μg/ml, MAb D3114/63 was diluted 1:40, and the omniserum and anti-R36A were diluted 1:50 for assaying the spinal fluids. + or −, Presence or absence of antigen detected by the antibody, respectively.
[b] The percentage of culture-positive pneumococcal spinal fluids which were found to be positive by the dot blot assay.
[c] The percentage of culture-negative pneumococcal spinal fluids which were found to be negative by the dot blot assay.
[d] The percent agreement between positive and negative culture results and results with the dot blot assay.

ELISA format as described above. The percent inhibition was calculated by dividing the optical density at 405 nm ($OD_{405}$) value of the test sample by the control (50 μl of PBS instead of spinal fluid) $OD_{405}$ value and multiplying by 100.

## RESULTS

The results of the dot blot assay for the detection of pneumococcal antigen in spinal fluids are shown in Table 2. Anti-PC antibody HPCM2 detected 72.7% of the culture-positive pneumococcal spinal fluids. HPCM2 only detected one false-positive result out of 45 culture-negative pneumococcal spinal fluids. This single false-positive spinal fluid was from a case of meningitis in which *N. meningitidis* was isolated.

The D3114/63 antibody detected 68.2% of the pneumococcal culture-positive spinal fluids and one false-positive fluid (Table 2). The single false-positive spinal fluid was the same one detected by the HPCM2 MAb.

The omniserum detected 72.7% of the pneumococcal culture-positive spinal fluids and one false-positive fluid (Table 2). The single false-positive spinal fluid was from a case of group B streptococcal meningitis.

Anti-R36A detected 86.4% of the pneumococcal culture-positive spinal fluids but detected 26 false-positive fluids (57.8%) (Table 2). False-positive spinal fluids detected by this antibody included 11 of 20 *H. influenzae* type b, 5 of 7 *N. meningitidis*, 4 of 7 group B streptococci, 5 of 5 other bacteria, and 1 of 6 control spinal fluids.

Two of the 22 culture-positive pneumococcal spinal fluids were not detected as positive by any of the six antibodies, and two others were only detected by the anti-R36A sera.

To compare the effectiveness of IgG versus IgM anti-PC MAbs, each anti-PC MAb was diluted to 10 μg/ml and used to test six random pneumococcus-positive and four random pneumococcus-negative spinal fluids as well as the level of detecting PC-BSA and live and heat-killed pneumococci (Table 3). The MAb 59.6C5 was slightly better than 140.1C2, but HPCM2 was more sensitive than either of the other two. Based on these results, the difference in the ability to detect positive pneumococcal spinal fluids between the anti-PC antibodies appeared to be due to isotype such that IgM > IgG3 > IgG2b.

The level of pneumococcal antigen detected by each of the antibodies was determined in both the dot blot assay and the ELISA (Table 4). HPCM2 detected as few as $10^7$ live or heat-killed pneumococci per ml and detected <20 μg of PC-BSA per ml and 2 μg of PC-BSA per ml by the dot blot assay and ELISA, respectively. D3114/63 detected $10^6$ live and $10^8$ heat-killed pneumococci per ml by the ELISA. The omniserum detected as few as $10^7$ live or heat-killed pneumococci per ml by the dot blot or the ELISA. Anti-R36A

TABLE 3. Determination of isotype importance in detecting pneumococcal meningitis by anti-PC MAbs with the dot blot assay[a]

| MAb isotype | No. with pneumococcal culture | | % Sensitivity | % Specificity | % Agreement | Level of detection[b] | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | PC-BSA (μg/ml) | Pneumococci (CFU/ml) | |
| | + | − | | | | | Live | Heat killed |
| IgG2b + | 4 | 1 | 67 | 75 | 70 | 87 | $6 \times 10^7$ | $6 \times 10^7$ |
| − | 2 | 3 | | | | | | |
| IgG3 + | 4 | 2 | 80 | 60 | 70 | 353 | $6 \times 10^7$ | $>10^8$ |
| − | 2 | 2 | | | | | | |
| IgM + | 5 | 0 | 83 | 100 | 90 | 87 | $6 \times 10^7$ | $6 \times 10^6$ |
| − | 1 | 4 | | | | | | |

[a] The IgG2b, IgG3, and IgM anti-PC MAbs used were 140.1C2, 59.1C5, and HPCM2, respectively. All antibodies were assayed at 10 μg/ml. + or −, Presence or absence of antigen detected by the antibody, respectively. See footnotes *b*, *c*, and *d* of Table 2.
[b] Minimum amount of protein or number of live or heat-killed pneumococci detected by the respective anti-PC MAbs. Fifteen microliters of each solution was assayed.

TABLE 4. Level of pneumococcal antigen detected by four antibodies with the dot blot assay and the ELISA[a]

| Assay | Antibody | Pneumococcal antigen (CFU/ml) | | | PC-BSA (μg/ml) |
|---|---|---|---|---|---|
| | | Live | Heat-killed | Lysate | |
| Dot blot | HPCM2 | $2 \times 10^7$ | $1 \times 10^7$ | 4.0 | <20 |
| | D3114/63 | $>10^8$ | $7 \times 10^7$ | >373 | >853 |
| | Omniserum | $2 \times 10^7$ | $1 \times 10^7$ | 4.0 | >853 |
| | Anti-R36A | $2 \times 10^6$ | $7 \times 10^5$ | 0.4 | 126 |
| ELISA | HPCM2 | $1 \times 10^7$ | $1 \times 10^7$ | 0.9 | 2 |
| | D3114/63 | $1 \times 10^6$ | $1 \times 10^8$ | 250 | >853 |
| | Omniserum | $2 \times 10^7$ | $2 \times 10^7$ | 4.7 | >853 |
| | Anti-R36A | $7 \times 10^5$ | $7 \times 10^5$ | 0.2 | 7 |

[a] Values represent the minimum number of pneumococci or amount of protein detected by the respective assay. The dot blot assay used 15 μl of solution, and the ELISA used 100 μl of solution.

antiserum detected $10^6$ live and heat-killed pneumococci per ml by the dot blot assay. The D3114/63 and omniserum antibodies did not react with PC-BSA, as would be expected.

Studies to determine the feasibility of shortening the assay time were conducted by using an ELISA format with heat-killed pneumococci as the antigen. The concentrations of antibody and conjugate were varied with the length and temperature of incubation to determine the conditions necessary to run the assay in the shortest possible time without compromising sensitivity (Table 5). By diluting a positive ELISA reaction and comparing visual assessment with spectrophotometric values at 405 nm, we found that an $OD_{405}$ of 0.3 or greater was sufficient to visually distinguish a positive reaction from a negative reaction. The data indicate that using an $OD_{405}$ of 0.3 as an endpoint, the test could be run in less than 60 min if 8 μg of the HPCM2 antibody per ml and 2 μg of the enzyme-labeled conjugate per ml were used and if the incubation times were 15 min for both the antibody and conjugate and 10 min for the substrate; each incubation was at 37°C.

The possibility that an inhibition assay would provide a more rapid test system was evaluated by using heat-killed pneumococci as the solid-phase antigen. Randomly selected positive and negative serially diluted spinal fluids were mixed with the screening antibody in the microtiter wells coated with heat-killed pneumococci. The percent inhibition necessary to separate positive and negative samples was selected to be ≥20% for all the antibodies except HPCM2, where ≥40% inhibition was chosen (Table 6). The reason for selecting an inhibition of ≥40% for HPCM2 is evident from Fig. 1, which shows that the percent inhibition of HPCM2 binding by all of the spinal fluids was higher than the percent inhibition of binding with the other antibodies. The inhibition assay with HPCM2 successfully predicted five culture-negative and five culture-positive pneumococcal spinal fluids; one of the positive fluids was one not detected by the dot blot assay. D3114/63 detected four of five positive fluids (the one missed was also not detected by the dot blot assay) but gave three false-positive reactions. Omniserum detected four of five positive fluids but gave two false-positive reactions. Anti-R36A antiserum detected only two of five positive spinal fluids and one false-positive reaction.

A survey of pneumococci and other gram-positive and gram-negative bacteria showed that IgM anti-PC MAbs react with the lysates and/or colony blots of all of 300 pneumococcal isolates tested. The MAb D3114/63 reacted with all of 79 pneumococcal isolates by the colony blot assay and with 93% of pneumococci by lysate blotting. Both anti-PC and D3114/63 antibodies were highly specific. With the exception of two weak reactions for the anti-PC MAbs with *N. meningitidis*, the anti-PC and D3114/63 MAbs did not react with any of 62 isolates of other bacteria including group A, B, C, D, and G and viridans group streptococci, *L. monocytogenes*, *H. influenzae*, *N. meningitidis*, and *Escherichia coli*.

## DISCUSSION

Of the antibodies tested, the IgM anti-PC MAb and the omniserum gave comparable results. They both detected 73% of the pneumococcal culture-positive spinal fluids and detected only 2% of the spinal fluids that were either normal or were culture positive for an organism other than the pneumococcus. Omniserum detected one pneumococcus-positive spinal fluid that HPCM2 missed, but HPCM2 detected one fluid that omniserum missed. Thus, although the anti-PC MAb did not perform better than the omniserum, it should be much easier to produce in large quantities for use in standardized assays.

Anti-PC antibodies of three different isotypes, IgM, IgG2b, and IgG3, were tested. Comparisons of these antibodies demonstrated greater sensitivity of IgM than IgG antibodies. This difference is undoubtedly the result of the isotype, because these MAbs are all of the T15 idiotype and have been shown to have essentially identical binding sites (1). The greater efficiency of the IgM over the IgG antibodies is probably the result of the higher valence of IgM as opposed to IgG antibodies.

The D3114/63 was as specific as the IgM anti-PC antibody and detected only one less pneumococcal culture-positive spinal fluid. D3114/63 reacts with a different determinant on the pneumococcal C carbohydrate than does the anti-PC antibody (13). Although D3114/63 is not superior to the anti-PC antibodies for the detection of pneumococcus-infected spinal fluids, it may be useful along with anti-PC antibodies for the identification of pneumococci isolated from patients. Together the two MAbs detected 82% of the culture-positive spinal fluids.

TABLE 5. Antibody and conjugate titration to determine optimal concentration and incubation[a]

| Concn (μg/ml) | | Incubation time (min) for conjugate | $OD_{405}$ for antibody incubation time (min): | | |
|---|---|---|---|---|---|
| Conjugate[b] | Antibody[c] | | 15 | 30 | 45 |
| 2 | 8 | 15 | 0.443 | 0.488 | 0.519 |
| | | 30 | 0.830 | 0.834 | 0.854 |
| | | 45 | 0.878 | 0.989 | 1.136 |
| 2 | 4 | 15 | 0.395 | 0.452 | 0.566 |
| | | 30 | 0.648 | 0.718 | 0.952 |
| | | 45 | 0.918 | 1.089 | 1.314 |
| 1 | 8 | 15 | 0.245 | 0 | 0.284 |
| | | 30 | 0.469 | 0 | 0.462 |
| | | 45 | 0.630 | 0 | 0.654 |
| 1 | 4 | 15 | 0.247 | 0 | 0.286 |
| | | 30 | 0.386 | 0 | 0.443 |
| | | 45 | 0.664 | 0 | 0.740 |

[a] Solid-phase antigen was $10^7$ heat-killed R36A per well. Incubation was at 37°C. The substrate was incubated for 10 min and the reaction was read as $OD_{405}$.
[b] Alkaline phosphatase-labeled goat anti-mouse immunoglobulin.
[c] Anti-PC MAb HPCM2.

TABLE 6. Detection of pneumococcal antigens in spinal fluids of patients with meningitis by four antibodies based on an inhibition ELISA[a]

| Antibody | % Inhibition | No. with pneumococcal culture | | % Sensitivity | % Specificity | % Agreement |
|---|---|---|---|---|---|---|
| | | + | − | | | |
| HPCM2 + | ≥40 | 5 | 0 | 100 | 100 | 100 |
| − | | 0 | 5 | | | |
| D3114/63 + | ≥20 | 4 | 3 | 80 | 40 | 60 |
| − | | 1 | 2 | | | |
| Omniserum + | ≥20 | 4 | 2 | 80 | 40 | 70 |
| − | | 1 | 3 | | | |
| Anti-R36A + | ≥20 | 2 | 1 | 40 | 80 | 60 |
| − | | 3 | 4 | | | |

[a] See footnotes of Table 2.

Four of the pneumococcal culture-positive spinal fluids were not detected by the anti-PC, D3114/63 antibodies, or omniserum. Presumably the antigen concentration of these spinal fluids was lower than the level of detection. Studies quantitating the level of pneumococci in spinal fluid have demonstrated a wide range of antigen levels. Feldman (5) found in five infants with pneumococcal meningitis a range of 450 to $2.6 \times 10^7$ pneumococci per ml of spinal fluid (mean, $3.87 \times 10^5$ pneumococci per ml). Olcen (14) found concentrations of $<10$ to $3 \times 10^7$ pneumococci per ml of spinal fluid (mean, $8 \times 10^5$ pneumococci per ml). Greenwood et al. (8) studied the levels of pneumococci in 28 adults with meningitis and found a range of $2 \times 10^6$ to $1 \times 10^9$ pneumococci per ml.

The false-negative spinal fluids in our study may have been taken early in the infection before sufficient antigen concentrations had developed for detection. Our assays to determine the level of antigen detected by the antibodies under the defined conditions of this study indicate that $10^7$ live or heat-killed pneumococci per ml, 4 μg of lysate protein per ml, or 2 μg of PC-BSA per ml is necessary for positive tests with the different assay procedures using the anti-PC

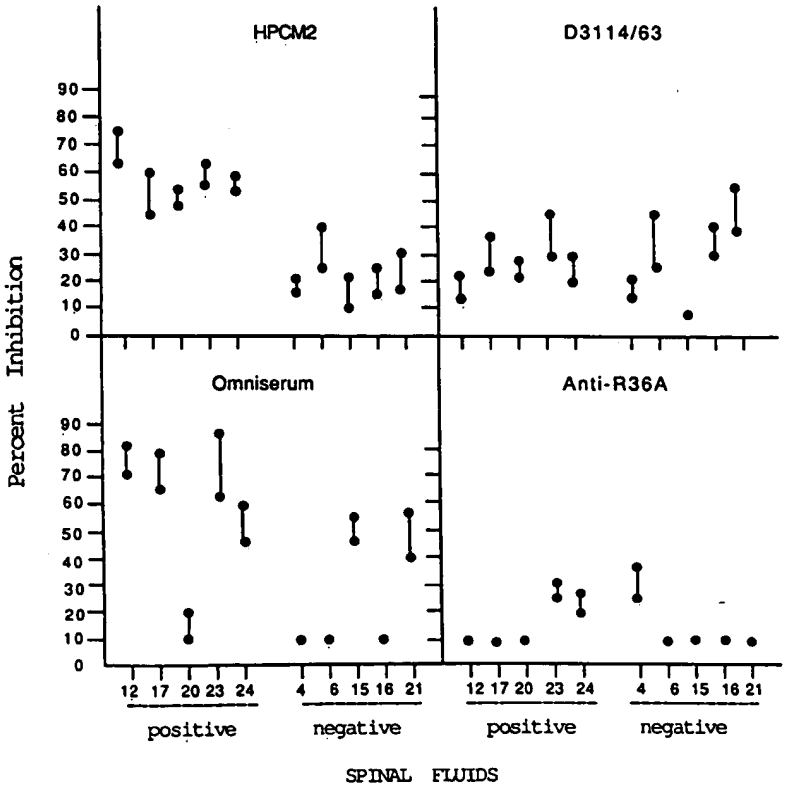



FIG. 1. Percent inhibition by five pneumococcal culture-positive and five culture-negative spinal fluids tested by an ELISA with four antibodies. Duplicate results of each test are shown.

MAb, HPCM2. These antigen concentrations are within the range normally found in pneumococcal meningitis as described above. Various pneumococcal antigen preparations were tested, since the form of the pneumococcal antigen (whether particulate or soluble) in spinal fluid is unknown.

Although anti-R36A antibody was the most sensitive, it is not specific enough to be useful as a screening reagent. However, with suitable absorption such an antiserum might react more specifically with pneumococcal antigens.

A direct ELISA was used to provide a quantitative assay to determine the conditions that would yield a useful result in the shortest possible time. We found that by adjusting the concentrations of the antibody and conjugate the assay could be completed with 15-min incubations for both antibody and conjugate followed by a 10-min incubation with the substrate. The assay time could probably be shortened further by directly conjugating the anti-PC antibody to alkaline phosphatase, thereby eliminating one of the incubation steps. If a similar protocol could be developed for the dot blot assay, this type of modification would be important for the development of an assay that would be useful in a clinical setting.

The inhibition ELISA was examined as an alternative diagnostic assay, since an inhibition format would circumvent the initial absorption step of binding the spinal fluid to the plate necessary in a conventional direct ELISA. The results indicated the feasibility of this method with the HPCM2 anti-PC antibody.

Sorensen (15) found that anti-PC MAbs would bind to beta-lipoprotein in serum and spinal fluids and concluded that these antibodies could not be used for the detection of PC-containing antigens in these fluids because of nonspecific reactions. Our results do not show any problems with nonspecific reactions among the anti-PC MAbs tested. Possibly the difference in the assay technique employed may explain the nonspecific reactions.

Overall, we feel that our results are quite significant because they indicate that, at least in this assay system, readily available IgM anti-PC MAbs are as useful as a mixture of polyclonal antisera for the detection of pneumococcal antigens in biologic samples. IgM antibodies with essentially identical specificity to the IgM anti-PC antibody that we have used have been frequently produced in several laboratories (1, 6). With proper care of the cell lines it should be much easier to provide these MAbs than the mixture of immune sera currently in use in most pneumococcal diagnostic assays.

## ACKNOWLEDGMENTS

We are grateful for the advice and encouragement from Hugh Dillon and Deborah Talkington.

This study has been supported by Public Health Service grants AI 18557, HD 17812, and AI 07051-11 from the National Institutes of Health and by Alabama Research Institute grant 85-06.

## LITERATURE CITED

1. **Andres, C. M., A. Maddalena, S. Hudak, N. M. Young, and J. L. Claflin.** 1981. Anti-phosphocholine hybridoma antibodies. II. Functional analysis of binding sites within three antibody families. J. Exp. Med. **154**:1584–1598.
2. **Briles, D. E., C. Forman, S. Hudak, and J. L. Claflin.** 1984. The effects of subclass on the ability of IgG anti-phosphocholine antibodies to protect mice from fatal infection with *Streptococcus pneumoniae*. J. Mol. Cell. Immunol. **1**:305–309.
3. **Colding, H., and I. Lind.** 1977. Counterimmunoelectrophoresis in the diagnosis of bacterial meningitis. J. Clin. Microbiol. **5**: 405–409.
4. **Drow, D. L., D. F. Welch, D. Hensel, K. Eisenach, E. Long, and M. Slifkin.** 1983. Evaluation of the Phadebact CSF test for detection of the four most common causes of bacterial meningitis. J. Clin. Microbiol. **18**:1358–1361.
5. **Feldman, W. E.** 1977. Relation of concentrations of bacteria and bacterial antigen in cerebrospinal fluid to prognosis in patients with bacterial meningitis. N. Engl. J. Med. **296**:433–435.
6. **Gearhart, P. J., N. D. Johnson, R. Douglas, and L. Hood.** 1981. IgG antibodies to phosphorylcholine exhibit more diversity than their IgM counterparts. Nature (London) **291**:29–34.
7. **Gray, B. M., and H. C. Dillon, Jr.** 1986. Clinical and epidemiological studies of pneumococcal infection in children. Pediatr. Infect. Dis. **5**:201–207.
8. **Greenwood, B. M., M. Hassan-King, P. G. Cleland, J. T. MacFarlane, and H. N. Yahaya.** 1986. Sequential bacteriological findings in the cerebrospinal fluids of Nigerian patients with pneumococcal meningitis. J. Infect. **12**:49–56.
9. **Holmberg, H., D. Danielsson, J. Hardie, A. Krook, and R. Whiley.** 1985. Cross-reactions between α-streptococci and omniserum, a polyvalent pneumococcal serum, demonstrated by direct immunofluorescence, immunoelectroosmophoresis, and latex agglutination. J. Clin. Microbiol. **21**:745–748.
10. **Klein, J. O.** 1981. The epidemiology of pneumococcal diseases in infants and children. Rev. Infect. Dis. **3**:246–253.
11. **Lund, E., A. Munksgaard, and S. M. Steward.** 1972. A new pneumococcus type. Type 47A. Acta Pathol. Microbiol. Scand. Sect. B **80**:497–500.
12. **Lund, E., and P. Rasmusen.** 1966. Omniserum. A diagnostic pneumococcus serum reacting with the 82 known types of pneumococcus. Acta Pathol. Microbiol. Scand. **68**:458–460.
13. **McDaniel, L. S., W. D. Waltman II, B. Gray, and D. E. Briles.** 1987. A protective monoclonal antibody that reacts with a novel antigen of pneumococcal teichoic acid. Microbiol. Pathogen. **3**: 249–260.
14. **Olcen, P.** 1978. Serological methods for rapid diagnosis of *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae* in cerebrospinal fluid: a comparison of coagglutination, immunofluorescence, and immunoelectroosmophoresis. Scand. J. Infect. Dis. **10**:283–289.
15. **Sorensen, U. B. S.** 1986. Monoclonal phosphorycholine antibody binds to beta-lipoprotein from different animal species. Infect. Immun. **53**:264–266.
16. **Tilton, R. C., F. Dias, and R. W. Ryan.** 1984. Comparative evaluation of three commercial products and counterimmunoelectrophoresis for the detection of antigens in cerebrospinal fluid. J. Clin. Microbiol. **20**:231–234.
17. **Wilson, C. B., and A. L. Smith.** 1986. Rapid tests for the diagnosis bacterial meningitis. Curr. Top. Infect. Dis. **7**:134–156.